Mining Wild Barley for Powdery Mildew Resistance

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Dedication

To my family, who was there when I needed a listening ear.
To Jo, who was always there with a good joke.
To Holly, who bolstered thesis-writing morale and helped me through finishing this degree.
To my Colleagues in the Applied Plant Science department, who helped me understand that it's not just about growing food, but who you share the meal with.

Abstract

Powdery mildew, caused by *Blumeria graminis* f. sp. *hordei (Bgh*) is a worldwide disease problem on barley (*Hordeum vulgare*) with severe impact on yield. Historically, resistance genes have been identified chiefly from cultivated lines and landraces; however, wild barley (*Hordeum vulgare* ssp. *spontaneum*) accessions have proven to be extraordinarily rich sources of pathogen resistance, including powdery mildew resistance genes. This study describes the characterization of a collection of 316 wild barley accessions, known as the Wild Barley Diversity Collection (WBDC), for resistance to powdery mildew and the genetic location of powdery mildew resistance loci. The WBDC was phenotyped for reaction to 40 different *Bgh* isolates at the seedling stage and then genotyped with three different marker sets: 3,072 Single Nucleotide Polymorphisms, 600 Diverse Array Technology (DaRT) markers, and 8,616 Diverse Array Technology-Sequencing (DArT-Seq) markers. Resistance in the WBDC to these isolates was distributed across a wide geographic range from North Africa in the west, throughout the Fertile Crescent region, and east to Central Asia. Accessions resistant to all 40 isolates of *Bgh* were not found; however, 52 accessions exhibited resistance to 90% (20 of 40) of the *Bgh* isolates. These results indicate that the WBDC is a rich source of powdery mildew resistance. Gene postulation analyses revealed that many accessions, while resistant, contained none of the 12 genes present in the Pallas near-isogenic lines Mla1, Mla3, *Mla6*, *Mla7*, *Mla9*, *Mla12*, *Mla13*, *Mlk1*, *MlLa*, *Mlg*, *Mlat*, and *Ml(Ru2)* based on infection type comparisons to the *Bgh* isolates. This result suggests that the accessions carry novel genes or gene combinations. In addition to the phenotypic analysis, we conducted a genome-wide association study (GWAS) of powdery mildew resistance in

the WBDC. Significant marker-trait associations were found for all marker types at nineteen different loci across the barley genome encompassing all chromosomes except 1H. Six of these loci have not been previously associated with powdery mildew resistance. These marker-trait associations will be useful for incorporating powdery mildew resistance into barley breeding programs.

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Chapter One:

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Introduction

Powdery mildew of barley (*Hordeum vulgare* L.), caused by *Blumeria graminis* DC f. sp. *hordei* Ém. Marchal*, (Bgh)* (Jørgensen, 1988; Bélanger *et al.*, 2002; Both & Spanu, 2004) is a common and damaging fungal disease. It is a problem in most major barley cultivation areas (Bélanger *et al.*, 2002; Brown & Hovmøller, 2002; Dean *et al.*, 2012). In Europe, it is one of the most common diseases on both winter and spring barley (Dreiseitl, 2011b). Yield losses due to powdery mildew can reach up to 20% in Europe, 30% in North Africa (Scott & Griffiths, 1980; Czembor & Czembor, 1998, 1999; Czembor, 2000a), and 40% in western Australia (Chaure *et al.*, 2000). One common method of control for powdery mildew is the use of fungicides, but such applications result in additional costs to producers and also possible environmental issues (Senesi & Miano, 1995; Verweij *et al.*, 2009; Komárek *et al.*, 2010). Moreover, the pathogen can develop resistance to these compounds after widespread agricultural use (Dekker, 1976; Brent *et al.*, 1998). Thus, the most effective and environmental-friendly approach to powdery mildew control is through the deployment of resistant cultivars.

Life cycle of Blumeria graminis

The haploid asexual spores (conidia) of *Blumeria graminis* are wind-dispersed, which are preferentially produced on exposed flat surfaces of plants, in particular the leaf surfaces (Bélanger *et al.*, 2002). Moderately ambient relative humidity facilitates infection, but is not strictly necessary since *Bgh* conidia are capable of germinating at 0 relative humidity, a trait unique to the fungus (Brodie, 1945; Manners & Hossain, 1963; Reuveni & Rotem, 1974; Quinti & Jr, 1982). In fact, extremely high humidity and

rainfall results in free water which has been observed to wash away conidiospores, resulting in lower colonization and dispersal rates (Reuveni & Rotem, 1974; Quinti & Jr, 1982). Initial infection of the plant begins with the release of enzymes from a conidium after it lands on a recognized surface. After approximately one hour, a primary germination tube (PGT) develops from the conidium and pierces the outer layers of the leaf, where it begins to absorb water and nutrients for subsequent use by the fungus (Edwards, 2002). An extracellular mycelial mat (the appressorial mat) helps anchor the conidium-PGT complex to the leaf surface, and 3-4 hours after infection a second appressorium and germ tube emerge from the conidium. This appressorium forms an infection peg, and if the plant does not respond with a hyper-oxidative or programmed cell death response, haustoria form inside the plant cell within 12 hours after initial infection. These haustoria absorb nutrients and feed further growth of the appressorial mat. Conidiophores grow from the appressorial mat, and conidia are produced within 4-6 days after initial infection (Deising, 2009)

The sexual stage of powdery mildew is less well understood. Ascospores form within cleistothecia: a brown-black pigmented spheroid with a two- to three-layer thick walled tissue surrounding an atrosclerocortex, subcortex, and ascospore laden hymenium. Cleistothesium are capable of remaining dormant on plant debris and during drought or extreme weather conditions, but are not as viable in the soil (Mathre, 1997). Under moist conditions, cleistothecia are softened and crack open, allowing the eventual ejection of ascospores, the primary source of inoculum in many production areas. Once these initial ascospore infections occur, the pathogen is capable of generating many secondary cycles

of infection via conidia. Conidia (and the mycelium) are capable of surviving in mild conditions on host plant material, so long as the host continues to live (Johnston, 1974).

Assessment of powdery mildew reaction

Powdery mildew is an obligate biotroph, and as such is difficult to isolate and grow on artificial media. This attribute makes the disease difficult to store, and requires specialized facilities to isolate and reproduce the fungus without contamination. With respect to the phenotyping, the interaction of powdery mildew on its barley host is one of the most important and widely used methods. By inoculating the pathogen onto a special set of barley genotypes each carrying a single resistance gene in a uniform background (i.e. near-isogenic lines or NILs), one can robustly determine the virulence pattern of individual isolates and therefore their genotype for avirulence loci given that this is a haploid fungus. Originally, 14 'Pallas' NILs that carried single resistance genes including *Mla8*, *Mla3*, *Mla9*, *Mla12*, *Mlc*, *Ml1402*, *Ml(Ru2)*, *Mlnn*, *Mlp*, Mlk *Mlat*, *mlo5*, *Mlh*, and *MlLa* were developed, but other isolines have since been developed and used. By inoculating uncharacterized barley germplasm with a diversity of *Bgh* isolates carrying different combinations of avirulence/virulence genes, one can postulate the putative powdery mildew resistance genes present in specific accessions by comparison with the reactions exhibited by each NIL. Knowing a pathogen's virulence spectrum allows for evaluation of new cultivars with selected known pathotypes (Torp *et al.*, 1978; Jensen *et al.*, 1992). New isolines are also frequently developed as new alleles are discovered, allowing for further differentiation of powdery mildew isolates (Dreiseitl *et al.*, 2006).

Powdery mildew resistance genes

mlo

The broad spectrum resistance gene *mlo,* which has been used extensively in European breeding programs (Jørgensen, 1993), is a defective allele that de-regulates programmed cell death and triggers host defense pathways (Büschges *et al.*, 1997). One of the pathways results in the development of cell wall appositions (CWAs) (Wolter *et al.*, 1993), which prevent penetration attempts (Bayles *et al.*, 1990) by infection pegs and appressorium of the fungus. Numerous *mlo* alleles have been isolated; however, the specific allele, *mlo11,* derived from three Ethiopian accessions (HOR2556, HOR1504, and HOR2937), is the most widely used in European breeding programs (Dreiseitl, 2013a; Jorgensen, 1992; Negassa, 2008). The gene *mlo* in all of its allelic forms has only been utilized in spring cultivars, as concurrent use in both winter and spring types weakens the protection that *mlo* provides (Schwarzbach *et al.*, 2002). *Bgh* is capable of adaptation to the partial protection that *mlo* provides in barley through the sexual recombination phase (cleistothecial stage) of its life cycle. In addition, use of *mlo* is associated to yield losses that are tied to the de-regulation of programmed cell death and consequent spontaneous necrosis of plant tissue (Brown, 2002). Some isolates of *Bgh* have been identified that have a high infection rate on barley accessions carrying *mlo* (Lyngkjaer *et al.*, 1995)

Mla

The *Mla* locus is a complex of multiple resistance (R) genes formed due to the duplicative nature of NBS-LRR genes (Wei *et al.*, 1999). The different alleles (e.g. *Mla6,*

Mla12, etc.) at the *Mla* locus function in a race-specific manner. Many breeding programs have focused on utilizing this highly diversified region of resistance genes in addition to the broad spectrum resistance locus *mlo*. The *Mla* alleles are characterized by a three-part system that consists of an N-terminal coiled-coil or TOLL/interleukin-1 receptor, a central nucleotide binding site, and a C-terminal leucine-rich repeat (LRR) (Shen & Schulze-Lefert, 2007). The key to resistance in plants is held in the hypervariable LRR region (DeYoung & Innes, 2006). These LRR regions are the recognition portion of the plant defense protein and consist of 20-29 amino acids that follow a β sheet form, often directly before an α helix (Kajava *et al.*, 1995). Part of the β strand contains a conserved segment of 11 amino acids (LxxLxLxxN/CxL, where x can be any amino acid, and L can be any hydrophobic amino acid)(Kobe & Kajava, 2001). The whole R gene product recognizes elicitors that are pathotype specific and trigger plant defenses, including but not limited to, hypersensitive cell death meant to isolate and deny the pathogen resources (Dangl & Jones, 2001). LRR regions have been shown in multiple systems to be under intensive diversifying selection pressure (Parniske *et al.*, 1997; Ellis *et al.*, 1999; Rose *et al.*, 2004; Dunning *et al.*, 2007). This kind of diversifying selection pressure has led to the hypothesis that R genes function as recognizers of non-self-structures. This consequently leads to a cycle of virulence and avirulence as plants and pathogens rapidly alter R and avirulence genes in response to changes in each other's gene composition (Wang *et al.*, 2007).

Other resistance genes

Other powdery mildew resistance genes have been identified (Jørgensen & Wolfe, 1994; Kintzios *et al.*, 1995; Dreiseitl *et al.*, 2007), but lack the extreme duplication and diversification that makes *Mla* such an effective source of resistance. These other major resistance genes include *Mlat, Mlk, Mlnn, Mlra, MlGa,* and *Mlp* found on chromosome 1H (Reviewed in Joergensen, 1994), *MlLa* on chromosome 2H (Hilbers *et al.*, 1992), *Mlg* on chromosome 4H (Görg *et al.*, 1993; Baker *et al.*, 1997), and *Mlh* on chromosome 6H (Jørgensen & Wolfe, 1994).

Sources of powdery mildew resistance

Modern cultivars and landraces

The gene pools for barley can be divided into three groups: a) the primary, consisting of domesticated barley (*Hordeum vulgare*) including advanced cultivars and lines, landraces, and also wild barley (*Hordeum vulgare* subsp. *spontaneum*); b) the secondary, which includes the single species of *Hordeum bulbosum*; and c) the tertiary, which includes more than 42 *Hordeum* species and subspecies (von Bothmer *et al.*, 2003). Most of the original sources of powdery mildew resistance genes came from domesticated cultivars in Europe (Kølster *et al.*, 1986; Brown & Jørgensen, 1991; Jørgensen & Wolfe, 1994). This was due to the fact that this continent had a conducive climate for the development of the disease (Hossain & Sparrow, 1991; Czembor & Czembor, 1999; Bonman *et al.*, 2005; Dreiseitl, 2013b). These domesticated sources of resistance, while easy to incorporate, had already undergone the bottleneck of

domestication and were limited in diversity. Thus, additional resistance sources were sought and found in non-European lines (Brückner, 1986).

Further surveys for powdery resistance genes were conducted in landrace collections (Moseman, 1955; Brückner, 1964, 1986; Wiberg, 1974; Hossain & Sparrow, 1991; Jørgensen & Jensen, 1997; Czembor & Czembor, 2002); however, these sources, while diverse, did not have a deep supply of resistance genes and effective resistance sources were largely exhausted (Czembor, 2000a). The mining of wild barley, secondary, and tertiary gene pools for new resistance genes has been more successful with the exhaustion of these post-domesticated sources (Dreiseitl, 2013a).

Wild barley

Hordeum vulgare ssp. *spontaneum* (wild barley) originates from the Middle East, an area with a high level of diversity in vertical zonality (the separation of climactic zones by altitude). This wide spectrum of conditions has led to diversification in terms of the species (von Bothmer *et al.*, 2003). Such conditions also favor the growth of *Bgh* (Dinoor & Eshed, 1990), encouraging development of host resistance through various co-evolutionary forces. Long-term examination of the *Bgh* evolutionary pressures encourage a diversity of virulence patterns as well (Wolfe & Schwarzbach, 1978; McDonald & Linde, 2002b; Brown & Tellier, 2011). This combination in terms of diversification of both host and pathogen creates a unique hotspot of diversity, which can be leveraged for a survey of resistance. This is not unique to the barley/*Bgh* host-pathotype system, and is a strategy known as Focused Identification of Germplasm Strategy (FIGS) which attempts to identify geographic regions that would produce desired phenotypes. This

strategy has been used successfully in identifying regions that would favor resistance to pathogens (Mackay & Street, 2004; Bonman *et al.*, 2005; El Bouhssini *et al.*, 2011; Filip Endresen *et al.*, 2011) as well as abiotic tolerance (Khazaei *et al.*, 2013)

Evaluations of wild barley germplasm have led to the identification of a number of resistance sources, some of them containing novel genes (Jahoor & Fischbeck, 1987a; Jahoor *et al.*, 1989; Dreiseitl & Bockelman, 2003; Dreiseitl *et al.*, 2007). These studies have identified new *Mla* variants, *Mlp,* and other loci that remain uncharacterized. From the evaluation of 116 Israeli and Jordanian accessions of wild barley, 70% were found to carry powdery mildew resistance (Fetch *et al.*, 2003). The Middle Eastern population of *Bgh*, as mentioned previously, is highly diverse due to the climactic conditions and diverse barley populations in the area. A larger collection of 1,383 accessions of wild barley were evaluated for powdery mildew resistance against 22 different pathotypes. Of these accessions, 123 showed at least some resistance to all pathotypes tested (Dreiseitl & Bockelman, 2003). One hundred and forty-one different accessions (121 of the accessions previously proven resistant plus 20 standards) were tested against 38 different isolates. In this evaluation, 134 of the accessions exhibited unique resistance combinations, with only one exhibiting resistance to all tested isolates (Dreiseitl & Dinoor, 2004). This study illustrates the diversity of the pathogens tested, as well as their importance in fully examining the resistance spectra of wild barley germplasm. Subsequent genetic studies on the most widely resistant accessions showed the presence of more than three genes controlling the resistance in this accessions, suggesting a rich resource for both allele and gene mining (Řepková *et al.*, 2006; Dreiseitl *et al.*, 2007a;

Repkova *et al.*, 2009; Řepková *et al.*, 2009; Teturová *et al.*, 2010; Řepková & Dreiseitl, 2010).

Additional testing (Dreiseitl *et al.*, 2006) was done using a differential set of isolines which contained at least 14 resistance genes derived from wild barley(Jahoor & Fischbeck, 1987a,b, 1993; Kintzios *et al.*, 1995; Schönfeld *et al.*, 1996). These NILs were tested against 97 isolates of *Bgh* that were collected from Har'el in central Israel. Virulence in this pathogen population was found for 10 of the 14 wild genes tested, indicating a diversity of pathotypes even for isolates collected from a small area. The NIL system was effective at detecting diversity and the study also highlighted the ability of the pathogen to differentiate among wild barley-derived resistance genes taken from one set of samples in a limited area during one year.

Secondary and tertiary gene pools

Evaluating the secondary and tertiary gene pools of *Hordeum* for powdery mildew resistance has been successful. *H. bulbosum,* the sole member of the secondary gene pool, has been evaluated for its potential in contributing powdery mildew resistance genes. Work identifying and introgressing resistance genes from *H. bulbosum* has been successful (Xu & Kasha, 1992; Pickering & Hill, 1995; Pickering & Steffenson, 1998; Pickering & Malyshev, 2000; Shtaya *et al.*, 2007). While introgression of genes from *H. bulbosum* is possible and has been done (Pickering, 2000), none of these efforts has translated into a commercially viable cultivar with powdery mildew resistance due to linkage drag and a lack of commercial interest in the secondary gene pool (Dreiseitl, 2013a). Additional germplasm sources in the tertiary gene pool, which consists of over

42 species and subspecies, have also been evaluated (von Bothmer *et al.*, 2003). Powdery mildew resistant accessions were reported in *Hordeum chilense, Hordeum marinum,* and *Hordeum nigirum*; however, difficulties in obtaining successful crosses have hampered the use of these germplasm resources for barley improvement (Andrivon & De Vallavieille-Pope, 1992; Rubiales *et al.*, 1993; Hernandez *et al.*, 2001; Taketa *et al.*, 2004; Gustaffson & Claesson, 2008).

Deployment of resistance in cultivars

Traditionally, introgression of resistance genes into European and Australian cultivars, has relied on identifying sources of resistance and backcrossing the resistance genes into modern varieties. These cultivars are targeted where there is the most need for resistance genes to powdery mildew, the most disease pressure, and the most agriculturally advanced infrastructure to support the deployment of cultivars. The R gene *mlo*, the most effective basal resistance gene currently in use, is restricted to only spring cultivars in order to prolong its effectiveness. The *mlo11* allele was the first major allele introduced into the first European lines and was sourced from an Ethiopian landrace. Resistance gene *mlo*9 was originally identified as a mutant in Diamont, and all commercial cultivars with this allele (such as cultivar "Alexis") are descendants of this mutant (Jørgensen, 1992). Alexis has been used extensively in European malting since its introduction in 1986 (von Bothmer *et al.*, 2003). In contrast to the spring type cultivars, winter type cultivars usually rely on a pyramid of multiple resistance genes (Hickey *et al.*, 2012; Dreiseitl, 2013b). These specific resistances include the multiple alleles at the *Mla* locus (*Mla6, Mla12, etc.*), as well as other loci, e.g. *Mlg, Mlh, MlRa, Ml(Ru)*

(Dreiseitl, 2013b). Many of these resistance genes were identified from old cultivars or from cultivated collections of barley that have not been surveyed with a large enough variety of powdery mildew pathotypes (Brückner, 1986; Jensen *et al.*, 1992; Czembor & Czembor, 1998).

Advanced backcross populations as a breeding tool

Many of the wild background traits are not agronomically desirable such as shattering and non-preferred flowering times (Pillen *et al.*, 2003; Yun *et al.*, 2006; Li *et al.*, 2006; von Korff *et al.*, 2006; Gyenis *et al.*, 2007; Schmalenbach *et al.*, 2009). The first powdery mildew resistance gene derived from wild barley was from accession "Voldagsen" carrying *Mla6* (Moseman & Jørgensen, 1973). This has been one of the more successful alleles from wild barley in European barley cultivation and is used commonly in both spring (Brown & Jørgensen, 1991) and winter barley types (Dreiseitl, 2013b). More recent advanced backcross (AB) schemes consist of wild barley accessions backcrossed to various cultivated barley parents. Several AB collections (Yun *et al.*, 2005; von Korff *et al.*, 2005, 2006; Schmalenbach *et al.*, 2008) have been evaluated for powdery mildew resistance. These advanced backcross collections serve as excellent adapted bridges between the diverse and novel genes contained within wild barley and the desirable domesticated traits of cultivated barley. Proper use of these collections relies on identifying Quantitative Trait Locus (QTL) and genes associated with resistance.

Genome wide association studies

The traditional approach to genetic mapping has been to associate genotype and phenotype data from segregating populations created from bi-parental crosses (Rafalski, 2002, 2010). This method takes advantage of genetic linkage and recombination to locate chromosomal segments that are associated with a trait of interest. Recently, genome-wide association studies (GWAS) have become popular due to the development of high-throughput genotyping methods. GWAS requires no progeny screening, crossing or population development. Association mapping uses correlation between the genotypes of a collection of germplasm and their related phenotypes to locate markers with resistance (Myles *et al.*, 2009; Painter *et al.*, 2011). This procedure can be used with any associated collection of germplasm to detect markers associated with any trait. Association mapping is advantageous for several reasons: (1) it provides higher information content per marker, as the markers are likely more polymorphic among members of a collection; (2) it increases the mapping resolution due to ancestral recombination in the association mapping panel; and (3) it eliminates the need to develop bi-parental mapping populations (Korte & Farlow, 2013). It however relies on linkage disequilibrium within the collection, and can fail in the presence of multiple genes of large effect that produce a given phenotype. GWAS has previously been utilized for mapping important traits in both cultivated and wild barley collections including: spot blotch resistance, stem rust resistance, leaf rust resistance, stripe rust resistance, fusarium head blight resistance, low temperature tolerance, protein fraction, starch content, thousand grain weight, plant height, heading date, drought tolerance, and malt quality, and spike architecture (Steffenson *et al.*, 2007; Massman *et al.*, 2010; Roy *et al.*, 2010; Wehner *et al.*, 2011; Ramsay *et al.*, 2011; von Zitzewitz *et al.*, 2011; Fettköther *et al.*, 2012; Wang *et al.*,

2012; Jin *et al.*, 2012; Pasam *et al.*, 2012; Berger *et al.*, 2013; Shu & Rasmussen, 2014; Pauli *et al.*, 2014; Muñoz-Amatriaín *et al.*, 2014; Mohammadi *et al.*, 2014).

The Wild Barley Diversity Collection

The Wild Barley Diversity Collection (WBDC) is a set of wild barley accessions collected primarily from the Fertile Crescent region of the Middle East (Steffenson et al., 2007). The WBDC is an expanded core of a collection originally assembled by Abdullah Jaradat. This collection was originally sourced from the International Center for Agricultural Research in the Dry Areas (ICARDA) wild barley genebank holdings, and was selected based on multiple ecogeographic factors (longitude and latitude, elevation, max/min temperature, rainfall and soil type). This germplasm set has three attributes that make it particularly useful: the accessions cover a wide geographic area and are subject to a range of ecological pressures that may facilitate the development of resistance, the accessions are genetically diverse, and the accessions have already been examined for other agronomic traits and disease resistance (Steffenson *et al.*, 2007; Roy *et al.*, 2010). Since these accessions have been evaluated against other pathogens as well, accessions can be chosen based on a multi-pathogen resistance score, and may offer more attractive options for breeders to incorporate multiple types of resistance in the same set of crosses.

Chapter Two:

Analysis of Resistance in the Wild Barley Diversity Collection to 40 Powdery Mildew Isolates

Introduction

Powdery mildew, caused by *Blumeria graminis* DC f. sp. *hordei* Ém. Marchal (*Bgh*)(Bélanger *et al.*, 2002; Both & Spanu, 2004), is a common and damaging fungal disease of barley (*Hordeum vulgare* L. subsp. *vulgare*) (Mathre, 1997; Chaure *et al.*, 2000) in many parts of the world. Yield losses in susceptible cultivars grown in conducive environments often range from 5-20%, but can reach as high as 40% (Chaure *et al*., 2000). One common method of control for powdery mildew is the use of fungicides, but such applications result in additional costs to producers and also possible environmental issues (Senesi & Miano, 1995; Verweij *et al.*, 2009; Komárek *et al.*, 2010). Moreover, resistance in the pathogen can develop to these compounds after widespread agricultural use (Dekker, 1976; Brent *et al.*, 1998). Thus, the most effective and environmentally-friendly approach to protecting the barley crop from powdery mildew infection is to develop and deploy resistant cultivars.

The most widely used powdery mildew resistance (R) genes in breeding programs include various alleles of *Mla* and *mlo*. The former is a complex nucleotide binding site-leucine rich region (NBS-LRR) locus on chromosome 1H that contains multiple R genes. These R genes are race-specific to various pathotypes of *Bgh* and form the *Mla* allelic series of *Mla1, Mla2, Mla3, Mla6, Mla12,* etc. (Joergensen, 1994; Wei *et al.*, 1999). The latter is a broad-spectrum R gene located on chromosome 4H. The *mlo* gene was originally identified in Ethiopian landraces, but was later isolated in several mutation studies of barley in multiple forms (Büschges *et al.*, 1997) as dysfunction of this gene may be caused in multiple ways. It has been highly effective in conferring resistance against powdery mildew in European barley lines for the past 45 years. Unfortunately, in

recent years, the *mlo*-based resistance has shown evidence of slowly eroding (Schwarzbach *et al.*, 2002). More than 80 other powdery mildew resistance genes have been described in barley (Jørgensen & Wolfe, 1994) with other novel genes identified in collections and bi-parental mapping populations (Pickering & Hill, 1995; Kinizios *et al.*, 1995; Schönfeld *et al.*, 1996; Backes *et al.*, 2003; Dreiseitl & Bockelman, 2003; Dreiseitl & Platz, 2012; Dreiseitl, 2013b). Some of the major genes more fully characterized and/or used in breeding include *Ml(Ru)*, *Mlat, Mlk, Mlnn, Mlra, MlGa,* and *Mlp* located on chromosome 1H (Jørgensen & Wolfe, 1994), *MlLa* on chromosome 2H (Hilbers *et al.*, 1992), *Mlg* on chromosome 4H (Görg *et al.*, 1993; Baker *et al.*, 1997), and *Mlh* on chromosome 6H (Jørgensen & Wolfe, 1994). Deployment of all these genes has been a major emphasis of breeding programs focused on developing powdery mildew resistance in barley. Unfortunately, race-specific resistance genes such as *Mla* have a shortcoming when deployed widely, resulting in selection pressure on the pathogen population to overcome the resistance (McDonald & Linde, 2002a). *Bgh* is capable of rapid changes that can overcome *Mla*-based resistance (Jahoor & Fishbeck, 1993). Sequencing analysis of three species of powdery mildew, including *Bgh,* revealed large-scale genome-size expansion, gene losses and retrotransposon proliferation. Examination of 248 candidate effector genes found less than 10 were conserved among the three species (Spanu *et al.*, 2010). Thus, there is a constant need to identify new genes that confer resistance to this pathogen.

Extensive powdery mildew evaluations of diverse *Hordeum* collections, including modern varieties, landraces, and also accessions of the wild progenitor of barley (*Hordeum vulgare* subsp. *spontaneum*), have been completed by a number of previous

researchers (Jahoor & Fischbeck, 1987a; Jahoor *et al.*, 1989; Legge *et al.*, 1998; Czembor & Johnston, 1999; Czembor, 2000b, 2001; Czembor & Czembor, 2002; Dreiseitl & Bockelman, 2003; Fetch *et al.*, 2003; Dreiseitl *et al.*, 2007; Steffenson *et al.*, 2007; Dreiseitl & Platz, 2012). Although these screening efforts have identified novel sources of resistance, there is always a need to continue evaluations of other unique *Hordeum* germplasm panels to different isolates of *Bgh*.

The Wild Barley Diversity Collection (WBDC) is a panel of *H. vulgare* subsp. *spontaneum* accessions assembled for exploiting allelic diversity for cultivated barley improvement. It consists of 318 accessions selected based on various ecogeographic factors (longitude and latitude, elevation, max/min temperature, rainfall and soil type) from across the range of the subspecies (Steffenson *et al*., 2007). Previously, the WBDC was used to identify accessions carrying spot blotch and leaf rust resistance and to position the resistance loci using an association mapping approach (Steffenson *et al.*, 2007; Roy *et al.*, 2010). The WBDC may also carry valuable powdery mildew resistance genes, but it has not been evaluated against various isolates of *Bgh*.

The objectives of this study were three fold: (1) characterize the infection phenotypes of WBDC accessions in response to 40 different isolates of *Bgh*; (2) examine the relationship between geographic origin of WBDC accessions and resistance to specific *Bgh* isolates; and (3) postulate the presence of specific resistance genes in the WBDC accessions.

Materials and Methods

Plant materials

The WBDC is comprised of 318 ecogeographically diverse accessions of *H. vulgare* subsp. *spontaneum* (Steffenson *et al*. 2007). Passport data and ecogeographic parameters for the collection sites of each accession are listed (Appendix Table 2.1). Twelve near-isogenic lines (NILs) (P01, P02, P03, P04B, P08B, P10, P11, P17, P23, P21, P20, P15) each carrying a single powdery mildew resistance gene (*Mla1*, *Mla3*, *Mla6*, *Mla7*, *Mla9*, *Mla12*, *Mla13*, *Mlk1*, *MlLa*, *Mlg*, *Mlat*, *Ml(Ru2)*, respectively) in the cultivar Pallas background were used to characterize the virulence phenotype of the *Bgh* isolates (Kølster *et al.*, 1986) and also for gene postulations in the WDBC.

Pathogen isolates

Forty selected reference isolates of *Bgh* held at the Agricultural Research Institute (ARI) in Kroměříž, Czech Republic were used to assay the resistance spectrum of the WBDC accessions. The *Bgh* isolates were characterized for virulence pattern (i.e. pathotype) based on their infection types (ITs) on the 12 Pallas NILs (Kølster *et al.*, 1986). Pathotypes were designated using a coded triplet system (Limpert & Muller, 1993), according to the following order of *Ml* resistance genes: *a1, a3, a6, a7, a9, a12, a13, k1, La, g, at* and (*Ru2)*. Before testing on the WBDC, each pathogen isolate was purified, verified for its virulence phenotype on 12 Pallas differential NILs, and increased on leaves of the susceptible line B-3213. To resolve as many individual resistance genes or combinations of genes as possible, a diverse collection of *Bgh* isolates was selected for this investigation. At least two isolates were virulent and avirulent on each of the known

Pallas lines. The number of resistance genes in the NILs overcome by this suite of isolates ranged from 1 (for isolate and pathotype 0004) to 12 (for isolate and pathotype 7777) (Table 1). Only two isolates had virulence on a single NIL, while the remainder exhibited virulence for multiple NILs (from 2 to 12). The 40 isolates keyed out to 37 different pathotypes.

Phenotyping protocol

All powdery mildew phenotyping experiments were conducted at the ARI. Forty to fifty seeds of each accession were sown in two (8 cm diameter) pots filled with a peat substrate (Rašelina Soběslav brand, Soběslav, Czech Republic) and kept in a mildew-proof greenhouse under normal daylight conditions. The screening tests were conducted from March to May in 2010. Leaf tissue was collected from these plants for all disease phenotyping experiments. Three leaf segments (2 cm long) from three different plants of each WDBC accession were cut from the central portion of the primary leaf (when the second leaf had just emerged) and placed with the adaxial side up in a Petri dish containing 0.8% water agar. Leaf senescence was inhibited with the addition of benzimidazole (40 mg^{-L}). For assays with individual isolates, a Petri dish with leaf segments (for each of the respective accessions) was placed at the bottom of an inoculation tower (Limpert, 1987). Inoculations were made by blowing spores by mouth from infected B-3213 plants onto uncovered Petri dishes containing the leaf segments. The approximate concentration of inoculum was eight conidia per mm^2 of leaf tissue. Each dish was then incubated at 18±2°C under artificial light conditions with a 12 hr photoperiod supplied by fluorescent lamps (30 ± 5 μmol $m^{-2}s^{-1}$).

Infection type scoring and data transformation

ITs, i.e. the interactions between specific host and *Bgh* genotypes, were evaluated eight and then 10-11 days after inoculation. ITs were assessed on the central part of the adaxial side of leaf segments using a 0-4 scale (Table 2) (Torp *et al.*, 1978). The raw ITs were assessed on each WBDC accession in response to the 40 different *Bgh* isolates (40 x 316 accessions=12,640 combinations). These data were collated to derive a frequency of resistance for each WBDC accession to the panel of powdery mildew isolates. The raw data scores were transformed before analysis of resistance was made.

The various combinations of raw powdery mildew ITs observed on leaves were transformed into final numeric values to more fully capture the variation observed in each specific interaction and to enable statistical analyses (Table 3). When the same IT (e.g. 0, 1, 2, 3, or 4) was observed across all three leaf segments in a particular interaction—as was the case in most instances—the respective integer of that IT was assigned for the final numeric value. Single IT observations given in parentheses, e.g. (2), indicate that the numbers of pustules observed on the adaxial surface of leaves were fewer than expected due to variation in the inoculation procedure or possibly the inherent low receptivity of the specific accession to infection by *Bgh*. Still, these low frequency ITs were clearly scorable and assigned the respective integers for the final numeric values (i.e. IT (1) was transformed into 1). In cases where a particular interaction exhibited two different ITs in roughly equal proportions on all three leaf segments (e.g. IT 1-2), the mean (1.5) of the two ITs was assigned as the final numeric value. When two ITs were not observed in equal proportion on all three leaf segments (e.g. IT 2(1) where the most predominant IT

was listed first and the minority type second and in parenthesis), the respective integer value of the most predominant IT was used for the final numeric value. In cases where there was a distinct IT difference observed across the three individual leaf segments of a specific interaction (i.e. those designated with a plus sign between infection types, e.g. IT 2+3), the final numeric value assigned was the respective integer of the first listed IT (i.e. 2) because it occurred on more leaves. For more complex IT combinations such as 0(4)+2 (indicating a predominance of IT 0 with a few 4 types on 2 of the three leaves, plus another leaf showing a different IT of 2), the final numeric value assigned was according to the most common overall IT observed--in this case a 0 as this was the most common IT observed over all the leaves. Other complex IT combinations were treated in a similar fashion. There were two cases where three different ITs were observed (1, 2, and 4 ITs on each on a separate leaf), and therefore the average of the ITs (2.33) observed was taken for the final numeric value, though it was rounded up to 2.5 so as to fit within the phenotypic observations of the population as a whole. The chief factors considered for the final numeric transformation of complex ITs was the importance of the most predominant IT and also simplification of the phenotype. These transformed ITs were used for all further phenotypic analyses, as well as the phenotypic data for a Genome Wide Association Study (GWAS) (Ames, Chapter 3). For analyses of the resistance spectrum, the final numeric data were categorized into three classes: 1=resistant (0-1.5 IT), 2=intermediate (2-2.5 IT) and 3=susceptible phenotype (3-4 IT), and these were used in Table 4. For the purpose of nomenclature, the 0-1.5 transformed ITs were regarded as indicative of host resistance in further analysis of the collection. In addition, an

aggregate score was created, which is the sum of all 40 IT scores for each accession. This score was used to examine overall resistance in the accessions.

Resistance spectrum

To more easily examine the similarity of resistance patterns among WBDC accessions and also to identify accessions with unique resistance spectra, a fourteen number octal code was created for each accession (Appendix Table 2.2). This octal code describes in a single numerical string the resistance spectrum of individual WBDC accessions to the forty *Bgh* isolates. Accessions with transformed infection types of 1.5 or lower were considered resistant, whereas those with >1.5 were considered susceptible. The octal coding system for this resistance spectrum of 40 isolates was assembled as listed: 0004, 0020, 0023, 0061, 0235, 0323, 0331, 0574, 1002, 1044, 1377, 1541, 2567, 3707, 3777, 4404, 4523, 4611, 4761, 4776, 5137, 5425, 5511, 5735, 5765, 5715, 6000, 6040, 6045, 6737, 7377, 7557, 7737, 7777, H-148, J-462, Q-301, S-016, Y-035, Y-069. Accessions with unique octal codes indicate the presence of a unique gene or combination of genes within the WBDC. This coding system was previously used to evaluate phenotypic diversity in a larger collection of wild barley (Dreiseitl & Dinoor, 2004)

Environmental factors

To test for native environmental factors that might be associated with the ITs of WBDC accessions to the 40 *Bgh* isolates, Spearman Rho correlation tests were performed (Spearman, 1904). Correlation tests were conducted between the transformed ITs

observed on WBDC accessions to the 40 *Bgh* isolates (i.e. the final numeric values) and several environmental factors (precipitation per year, max temperature per year, min temperature per year, and aridity) that were pulled from the International Center for Agricultural Research in the Dry Areas (ICARDA) logs for these accessions. In addition, the aggregate IT (as described above) was also used in similar correlation tests. Each test was a univariate test, only examining correlation between the IT scores of accessions to a single isolate and a single environmental variable.

The clustering patterns of resistance was also examined based on spatial proximity only with the aid of the Getis-Ord Gi method of statistical analysis (Getis & Ord, 1992; Ord & Getis, 1995) contained within the ArcGIS program (Johnston *et al.*, 2001; Wong & Lee, 2005). This method detects clusters of accessions that skew towards either a high or low score, and in this case would detect resistance or susceptibility hotspots. This method has been used previously in pattern analysis, and in particular is suitable for disease studies (Kelly-Hope *et al.*, 2009).

Postulation of powdery mildew resistance genes in WBDC accessions

To postulate the powdery mildew resistance genes present in the WBDC, we compared the transformed ITs of individual accessions to those exhibited by the 12 Pallas NILs across the suite of the 40 tested *Bgh* isolates. These comparisons were facilitated by the SAS module developed by Wamishe *et al.*, (2004). For these gene postulations, we considered accessions giving final numeric values of up to 2.5 as resistant, and used the reactions of the Pallas NILs as our cue for resistance. Low infection responses for the NIL lines were set at 1.5, 1.5, 1.5, 1.5, 1.5, 1, 1.5, 1.5, 2.5, 0, 2, 2.5 for *Mla1*, *Mla3*,

Mla6, *Mla7*, *Mla9*, *Mla12*, *Mla13*, *Mlk1*, *MlLa Mlg*, *Mlat*, *Ml(Ru2)*, respectively. These thresholds for resistance were taken from reactions on known resistance genes in Pallas isolines. Our reasons for setting these thresholds are that some known Pallas isolines contain genes that only contribute to partial resistance and do not reduce ITs to levels that we have previously defined as resistant (0-1.5), but instead reduce ITs to intermediate levels (2-2.5). To exclude R genes from a particular WBDC accession and obtain a short list of possible R genes carried in it, the program used data from every pathotype virulent on the accession to exclude all R genes that would contain resistance to that pathotype. Logically, an accession that is susceptible to a pathotype would contain none of the genes present in the NILs that would confer resistance to that pathotype. By examining all isolates that an accession is susceptible to, one can eliminate many of the NIL genes that could not be in that accession. From this logical elimination protocol, a short list of remaining putative R genes were obtained that could explain the resistance contained within individual accessions. The number of times genes were not eliminated by this program was obtained. This is only a list of candidate genes, and is not a declaration that all candidate genes are present in any given accession. In fact, in some cases, such as the presence of multiple *Mla* alleles, there is a limit of two alleles possible at the locus.

Results

Frequency and distribution of resistance in the WBDC

Forty *Bgh* isolates were tested on 316 of the 318 WBDC accessions, and the overall distribution of resistance in the accessions was examined (Appendix Table 2.2). Overall, 78.7% (249/316) of the accessions exhibited resistance to at least one of the isolates; 24.3% (77/316) were resistant to from two to five isolates; 9.8% (31/316) were resistant to six to ten isolates; 16.1% (51/316) were resistant to from 11 to 20 isolates; and 8.5% (27/316) were resistant to from 21 to 30 isolates. None of the accessions was resistant to all of the isolates. Accessions with the broadest spectrum of resistance (i.e. to >77% or 31/40 of the isolates) included WBDC accessions 021, 026, 037, 038, 042, 043, 053, 085, 089, 186, 188, 191, 248, 275, 289, 291, 346, 354. Notably, WBDC accessions 053, 085, and 089 had the broadest spectrum of resistance, giving low transformed ITs to 95% (38/40) of all tested isolates, except 0004 and 0323 (WBDC053), J-462 and Y-035 (WBDC085), 7737 and J-462 (WBDC089). Forty-eight accessions exhibited resistant (0-1.5) ITs to pathotype 7777, which carries virulence for all of the resistance genes present in the Pallas NILs (Table 4). This result suggests that these accessions carry resistance genes (or gene combinations) not present in the Pallas NILs. Twenty-one percent (67/316) of the accessions were susceptible to all of the isolates (Appendix Table 2.2). These accessions may not carry any R genes or ones not effective against the suite of isolates used in this study.

Geographical hotspots of resistance and susceptibility

To determine if there were any geographical regions that contain a preponderance of resistant accessions, we compared the geographic location of all WBDC accessions with their respective transformed ITs to each individual isolates and also to the aggregate IT scores across all isolates. No strong geographical trends were initially observed for resistance to individual isolates (data not shown) or across all isolates as given by aggregate IT scores (Figure 1).

To test for hotspots or clusters of WBDC accessions exhibiting resistance or susceptibility to each of the isolates, we used the Getis-ord Gi test within the ArcGIS package (McCoy, 2004). This test checks for correlation between proximal accessions, with the inverse square of the distance between accessions used as a testing factor. Variability for hotspot patterns were detected for the different isolates, although they were largely consistent with the aggregate hotspot patterns. Therefore, the aggregate score hotspots were examined to best summarize patterns. One important cluster of resistant accessions was found in southwestern Israel (some accessions showing significance at $p<0.05$, those in the center of the cluster $p<0.01$) near the cities of Ashdod and Ashkelon. In contrast, clusters of susceptible accessions were found in southwest (near Al Nabk) and northeast (near Al Qamishli) Syria, as well as a region in Central Asia (near Khujand, Tajikistan) (Figure 2). While the Getis-Ord Gi test is somewhat dependent on sample size (note the results of isolated accessions falsely showing hotspots), the hotspots identified here would be the least likely to be an artifact of the test, as there are many accessions in proximity to one another. The Getis-Ord Gi testing revealed patterns that were not readily discernable through casual inspection, due to the

mixture of IT scores exhibited by accessions in the region. While there are a skewed number of resistant accessions in the Ashdod and Ashkelon area, not all of the local accessions were resistant. While only the results from the aggregate scores were given (Figure 1), most of the IT score maps did show the small clusters in the same places shown in the aggregate score.

Environmental factors

To test for environmental factors that might be associated with the transformed ITs of WBDC accessions to the 40 isolates, Spearman Rho correlation tests were conducted (Spearman, 1904). While significant correlations were found (Table 5), the rho values were low, indicating that there were no strong environmental factors that could be used to predict the concentration of resistance in any locale.

Gene postulation indicates enrichment for some loci

To distinguish broad patterns of putative powdery mildew resistance genes present in the WBDC, an octal code was created for each accession in response to the group of 40 tested isolates. Overall, 64.5% (204/316) of accessions in the WBDC exhibited unique resistance spectra (Appendix Table 2.2), suggesting that they carry unique genes or gene combinations. Forty-five of these resistant accessions had identical octal codes, indicating that they carry the same or similar acting genes or gene combinations.

To postulate resistance genes in the WBDC, data from interactions with avirulent isolates were initially analyzed to eliminate genes that could not be present in an

accession given its reaction spectrum (Wamishe *et al.*, 2004). This analysis cannot discriminate when there are multiple resistance genes present in a single accession, but it was useful for establishing patterns of resistance in this collection, as well as characterizing the possible presence of resistance genes represented in the Pallas NILs. Again, for this test alone, accessions exhibiting transformed ITs ranging from 0 to 2.5 were classifiedas resistant (0-2.5) and those with ITs ranging from 3 to 4 were classified as susceptible (3-4). The reason for this is that some Pallas NILs exhibited ITs of 2 or 2.5, and would consequently be misclassified as susceptible under the original criteria. The results suggest that there are a large number of WBDC accessions that possibly carry the characterized genes of *Mla1* (77 accessions)*, Mla6* (80)*, Mla12* (74)*, Mlk1* (88)*, Mlat* (72), and *Ml(Ru2)* (80) (Table 6). *Mla13* is likely not present in the WBDC, and *Mla7*, *Mla9*, *MlLa* are candidates in only 2, 2, and 3 accessions, respectively. There is a positive trend between the aggregate pathotype score and the number of genes that were eliminated from the accession. However, there are some accessions that had all genes eliminated, but are still highly resistant (Appendix Table 2.2). This result suggests that these accessions carry powdery mildew resistance genes not represented in the set of Pallas NILs.

Discussion

Powdery mildew is a devastating disease in both spring and winter barley, resulting in 15-40% yield reductions during severe outbreaks (Brown *et al.*, 1991; Oerke & Steiner, 1994). Two of the key control measures for powdery mildew have been the deployment of resistance genes (in the form of both specific resistance genes and basal resistance genes) and use of fungicide treatments (Brent *et al.*, 1989; Wyand & Brown, 2005; Tucker *et al.*, 2013). With regulatory pressures on the use of fungicides and the increased interest in low input cropping systems, the development and deployment of resistant cultivars has taken on a new urgency. However, closely coupled with this research is a need to identify new effective powdery mildew resistance genes since the pathogen is highly variable and has a history of overcoming deployed R genes, especially if deployed singly. Diverse sources of disease resistance can often found in wild germplasm (Lehmann & Bothmer, 1988). The WBDC, a core collection of wild barley, has proven to be a rich source of resistance to stem rust and spot blotch (Steffenson *et al.*, 2007; Roy *et al.*, 2010) as well as many other diseases (B. Steffenson, unpublished). In this study, we examined the phenotypic responses of the WBDC to forty isolates of *Bgh* and identified accessions that conferred resistance to each of them. Our results support four main conclusions: 1) the WBDC is a rich resource for powdery mildew resistance, 2) resistant accessions can be identified across the geographical range of wild barley, 3) geographical hotspots were identified where resistance is abundantly found, and 4) certain putative resistance genes are found in high frequency in the WBDC.

The WBDC is a rich source of resistance to powdery mildew

The WBDC was previously shown to be rich source of resistance to stem rust, spot blotch, and leaf rust (Steffenson *et al.*, 2007). In this study, 251 of 316 total accessions were found to be resistant to at least one of the 40 *Bgh* isolates tested. Moroever, 206 unique resistance spectra were described from these 251 resistant WBDC accessions (Appendix Table 2.2). This result indicates that there are many diverse combinations of resistance genes within the collection. These results are in agreement with previous wild barley evaluations where ample numbers of highly resistant accessions were identified to powdery mildew (Jahoor & Fischbeck, 1987a, 1993; Kintzios *et al.*, 1995). Powdery mildew resistance in wild barley collections is more prevalent and diverse than that found in domesticated germplasm (Dreiseitl & Bockelman, 2003; Fetch *et al.*, 2003). The most widely resistant accessions found in this study were WBDC 053 (Baluchistan, Pakistan), 085 (Balqa, Jordan), and 089 (Amman, Jordan), which exhibited low ITs to 38 of the 40 isolates (Appendix Table 2.2). Each of these three accessions were susceptible to two different isolates, suggesting that they likely carry different resistance genes or combinations of genes. Fifty-two of the WBDC accessions were resistant to at least twenty of the *Bgh* isolates. There were at least ten accessions resistant to each isolate examined, indicating a possible diversity of resistance genes for use in breeding. This also means that resistance effective against all powdery mildew isolates used in this study could be theoretically be pyramided into a single line.

Results of the gene postulation analysis using the SAS program allowed for examination of patterns of resistance to each pathotype. There were distinct patterns in the ITs of accessions to particular isolates, indicating the possible presence of certain

resistance genes with unique signatures (Table 6). The *Mla6*, *Mla1*, *Mlk1*, *Mla12*, *Mlat*, *Ml(Ru2)* genes were postulated to be present in numerous accessions--sometimes in combination, even if they are unlikely to all be present due to the allelic nature of the *Mla* genes (i.e., *Mla6*, *Mla12,* and *Mla1* cannot all be present in the same accession, barring genome expansion). This result suggests that there are resistance genes or gene combinations in these lines that mimic the resistance spectra of the *Mla* alleles. In contrast, *Mla13* was not postulated to be present in the WBDC. Other patterns of resistance suggested the presence of *Mla7*, *Mla9*, *MILa*, but in low frequency.

Many of the widely resistant accessions found in this study apparently do not carry any of the R genes represented in the 12 Pallas NILs (Appendix Table 2.2). This is in agreement with other evaluations of wild barley germplasm (Czembor & Johnston, 1999; Negassa, 2008). It may be possible to resolve the identity of additional genes through comparisons with an expanded set of NILs (other isolines from the Pallas set are available and more have been developed from other backgrounds) and the same or perhaps an expanded set of *Bgh* isolates with different combinations of virulence/avirulence. The WBDC is therefore a rich source of powdery powdery mildew resistance genes that will prove useful in both future genetics and breeding efforts.

Resistance to powdery mildew is found across the entire habitat range of wild barley and exhibits hotspots

Significant positive and negative correlation coefficients were found between ITs exhibited on WBDC accessions and various environmental variables thought to impact the distribution and severity of powdery mildew; however, all of the r values from these

correlation tests were very low. Previous studies (Leur *et al.*, 1989; Karajeh, 2008) have shown that environmental influences are not significant enough alone to predict possible concentrations of resistance to *Bgh* in wild barley (Karajeh, 2008) and barley landraces (Leur *et al.*, 1989). In contrast, a survey of powdery mildew resistance in a collection of wild barley germplasm from Israel showed significant and appreciable environmental correlation. Number of rainy days and water availability were positively associated with resistance, whereas correlations with temperature were negatively associated with resistance (Nevo *et al.*, 1984). However, that study was performed with accessions collected from small geographical areas in Israel and may not be applicable in all geographical areas. The WBDC comes from a much broader geographical area and only contains six replicated geographic sites (i.e. where two or more accessions from the same GPS coordinate were tested), possibly resulting in the low correlations to environmental factors in comparison to the previous study. Ecological conditions that favor the host will also favor the pathogen, as they have both co-evolved in the same environment (Nevo *et al.*, 1984). Further tests were done with *H. vulgare* spp. *spontaneum* in Iran, Israel, and Turkey where temperature and humidity was implicated in genetic separation of sub-populations (Nevo *et al.*, 1986). That genetic separation is highly influenced by ecological conditions was also reported by Russell *et al.*, (2014). IT scores on WBDC accession were tallied according to their genetic sub-population, and marked differences were detected across subpopulations. In considering the recent data from Russell *et al*. (2014), there may be ecological factors that were not considered (such as monthly changes in temperature, aridity, rainfall, frequency of rainfall) in our study that may explain ecological conditions that favor resistance.

To examine the spatial clustering of accessions based on their IT scores, the Getis-Ord Gi hotspot analysis function was used. This analysis was done with both IT data from individual isolates as well as aggregate IT data. While only the results from the aggregate scores were given (Figure 1), most of the individual isolate geographic distributions did show clusters in the same sites as those revealed by the aggregate IT data. Many of the hotspots identified were for susceptibility, but there were also cases where clusters of resistance were clearly defined. However, one cannot draw any strong conclusions on whether any environmental parameters may have influenced the coevolution of host resistance in response to the pathogen. These hotspots of resistance provide meaningful guidance for future collection trips, perhaps using the Focused Identification of Germplasm Strategy (FIGS) (Mackay & Street, 2004; Khazaei *et al.*, 2013) in concert with WORLDCLIM data (Hijmans *et al.*, 2005) to more accurately pinpoint environmental factors that lead to powdery mildew resistance.

Utilization of the WBDC for developing cultivars with powdery mildew resistance

Our results clearly demonstrate that the WBDC is an excellent resource for powdery mildew resistance. A first step towards exploiting powdery mildew resistance in this collection will require mapping the resistance loci. One recent approach that has been widely adopted is the Genome Wide Association Study, or GWAS. Indeed, association mapping has been used on the WBDC to map resistance to spot blotch and stem rust (Steffenson et al., 2007; Roy et al., 2010). Thus, the phenotypic data presented here provide an excellent starting point for association mapping of powdery mildew resistance within the WBDC. Another approach is to develop and map loci in biparental

mapping populations developed from select, broadly resistant WBDC accessions. These can be in the form of recombinant inbred line (RIL) populations, doubled haploid (DH) populations or advanced backcross QTL (AB-QTL) populations (Tanksley & Nelson, 1996; Wang & Chee, 2010). These types of populations have been developed for wild barley accessions and used to map agronomic and disease resistance loci (e.g., Pillen *et al.*, 2003; von Korff *et al.*, 2005, 2006; Yun *et al.*, 2006; Li *et al.*, 2006; Schmalenbach *et al.*, 2008, 2009). For example, a RIL population was developed with accession WBDC 355 crossed to cv. Harrington and used to map resistance loci to multiple pathogens including powdery mildew (Yun *et al.*, 2005). From this work, two quantitative trait loci QTL were identified: one that coincided with the location of *Mla* on chromosome 1H and the other one close to the *Mlg* gene on chromosome 4H. Doubled Haploid (DH) populations were also developed with two other accessions from the WBDC, Shechem (WBDC 349) and Damon (WBDC 348), which were both crossed to cultivar Harrington. QTL mapping of the Shechem/Harrington and Damon/Harrington DH populations resulted in the identification of 17 and 15 QTLs for resistance to six powdery mildew isolates, respectively (Alsop, 2009). Three of the isolates used in that evaluation (0331, 7557, and 0574) were also used in this evaluation of the WBDC. For the Shechem/Harrington DH population, in response to 2001, 0024, 0331, 0574, 0666 and 7557; 3, 2, 3, 2, 3, and 4 QTLs were found respectively. For the same isolates in the Damon/Harrington DH population 3, 4, 2, 3, 1, and 2 QTLs were found respectively. The use of AB-QTL populations will result in the added benefit of introgressing the resistance loci into an elite background and eliminating many of the deleterious alleles from the wild barley accession. To facilitate this effort, 25 accessions were crossed with the elite

six-rowed malting cultivar Rasmusson and BC_2 breeding and mapping populations developed (Nice and Muehlbauer, unpublished results). Of these 25 populations, 20 of them show resistance to one or more powdery mildew isolates (WBDC302, WBDC092, WBDC336, WBDC142, WBDC227, WBDC016, WBDC173, WBDC35, WBDC150, WBDC061, WBDC035, WBDC182, WBDC234, WBDC292, WBDC032, WBDC340, WBDC042, WBDC348, WBDC082, and WBDC115). Of these, WBDC042 has the broadest resistance being susceptible to only isolates J-462, Q-301, Y-035, and Y-069. These populations are excellent resources for mapping and subsequently incorporating powdery mildew resistance into elite barley breeding germplasm, despite their small population size.

Table 1. List of the 40 powdery mildew isolates used in this study and their virulence phenotypes and pathotype designations based on the Pallas near-isogenic lines.

	Pallas Block	Block 1	Block 1	Block 1	Block 2	Block 2	Block 2	Block 3	Block 3	Block 3	Block 4	Block 4	Block 4
	NIL's Resistance Gene	*Mla1*	*Mla3*	*Mla6*	*Mla7*	*Mla9*	*Mla12*	*Mla13*	*Mlk1*	*MlLa*	*Mlg*	*Mlat*	*Ml(Ru2)*
Pathotype	Isolate												
0004	0004	--	--	--	--	--	--	--	--	--	--	--	+
0020	0020	--	--	--	--	--	--	--	+	--	--	--	--
0023	0023	--	--	--	--	--	--	--	+	--	+	+	--
0061	0061	--	--	--	--	--	--	--	+	+	+	--	--
0235	0235	--	--	--	--	+	--	+	+	--	+	--	+
0323	0323	--	--	--	+	+	--	--	+	--	+	+	--
0331	0331	--	--	--	+	+	--	+	+	--	+	--	--
0574	0574	--	--	--	+	--	+	+	+	+	--	--	+
1002	1002	+	--	--	--	--	--	--	--	--	--	+	--
1044	1044	+	--	--	--	--	--	--	--	+	--	--	+
1377	1377	+	--	--	+	+	--	+	+	+	+	+	+
1541	1541	+	--	--	+	--	+	--	--	+	+	--	--
2567	2567	--	+	--	+	--	+	--	+	+	+	+	+
3707	3707	+	+	--	+	+	+	--	--	--	+	+	+
3777	3777	+	+	--	+	+	+	+	+	+	+	+	+
4404	4404	--	--	+	--	--	+	--	--	--	--	--	+
4523	4523	--	--	+	+	--	+	--	+	--	+	+	--
4611	4611	--	--	+	--	+	+	+	--	--	+	--	--
4761	4761	--	--	+	+	+	+	--	+	+	+	--	--
4776	4776	--	--	+	+	+	+	+	+	+	--	+	+
5137	5137	+	--	+	+	--	--	+	+	--	+	+	+
5425	5425	+	--	+	--	--	+	--	+	--	+	--	+
5511	5511	+	--	+	+	--	+	+	--	--	+	--	--
5735	5735	+	--	+	+	+	+	+	+	--	+	--	+
5765	5765	+	--	+	+	+	+	--	+	+	+	--	+
5715	5715	+	--	+	+	+	+	+	--	--	+	--	+
6000	6000	--	+	+	--	--	--	--	--	--	--	--	--
6040	6040	--	+	+	--	--	--	--	--	+	--	--	--
6045	6045	--	+	+	--	--	--	--	--	+	+	--	+
6737	6737	--	+	+	+	+	+	+	+	--	+	+	+
7377	7377	+	+	+	+	+	--	+	+	+	+	+	+
7557	7557	+	+	+	+	--	+	+	--	+	+	+	+
7737	7737	+	+	+	+	+	+	+	+	--	+	+	+
7777	7777	+	+	+	+	+	+	+	+	+	+	+	+
5667	H-148	+	--	+	--	+	+	--	+	+	+	+	+
7467	J-462	+	+	+	--	--	+	--	+	+	+	+	+
(UNKNOWN)	Q-301	--	--	--	--	--	--	--	--	--	--	--	--
5466	S-016	+	--	+	--	--	+	--	+	+	--	+	+
5667	Y-035	+	--	+	--	+	+	--	+	+	+	+	+
7467	Y-069	+	+	+	--	--	+	--	+	+	+	+	+

[1]The + and -- indicates virulence and avirulence, respectively of the isolate for the resistance gene present in the Pallas near-isogenic line.
[2]Lines developed by Kølster *et al.* (1986)
Raw infection type data can be obtained from the Authors

Table 2. Criteria[1] used for classifying powdery mildew infection types on the Wild Barley Diversity Collection.

Infection type	Mycelium growth	Sporulation	Development of Chlorosis/Necrosis	General reaction
0	None	None	-	Resistant
0-1	None	None	+	Resistant
1	Weak	None	+	Resistant
1-2	Weak	Weak	+	Resistant
2	Moderate	Weak	+	Intermediate
2-3	Moderate	Moderate	+	Intermediate
3	Strong	Moderate	+	Susceptible
3-4	Strong	Strong	+	Susceptible
4	Strong	Strong	-	Susceptible

[1]Table taken from Torp *et al.* (1978)

Table 3. Translation of raw powdery mildew infection type data to transformed infection type data

Raw Infection Type Scores	Description of raw infection type scores	Transformed infection type values
X_1	Only a single IT was observed	X_1
X_1-X_2	Two different ITs were observed in roughly equal proportion	$((X_1+X_2)/2)$
(X_1)	Only a few colonies of a single IT were observed	X_1
(X_1+X_2)	Two different ITs were observed across the three individual leaf segments with X_1 observed on two leaf segments and X_2 on one leaf segment. Is suggestive of genetic heterogeneity in wild barley accession	X_1
$X_1(X_2)$	Two different ITs were observed on the same leaves with X_1 in the majority and X_2 in the minority	X_1
$(X_1+X_2+X_3)$	Three different ITs were observed, one on each of the three leaf segments	$((X_1+X_2+X_3)/3)$

[1]X here is an Infection Type (IT) (i.e. 0, 1, 2, 3, or 4)

Table 4. Percentages of accessions exhibiting resistance to each pathotype of *Blumeria graminis* f. sp. *hordei*.

Isolates	% Resistant[1]	% Intermediate[2]	% Susceptible[3]
1044	48.4% (153)	27.2% (86)	24.4% (77)
6000	42.4% (134)	27.8% (88)	29.7% (94)
0061	31.0% (98)	33.2% (105)	35.8% (113)
1541	31.0% (98)	35.4% (112)	33.5% (106)
4404	30.7% (97)	32.0% (101)	37.3% (118)
0023	30.4% (96)	41.8% (132)	27.8% (88)
1002	29.7% (94)	41.5% (131)	28.8% (91)
0004	28.8% (91)	36.7% (116)	34.5% (109)
0020	26.9% (85)	37.3% (118)	35.8% (113)
0574	26.3% (83)	38.6% (122)	35.1% (111)
0235	25.6% (81)	35.1% (111)	39.2% (124)
1377	23.7% (75)	40.2% (127)	36.1% (114)
0323	22.8% (72)	36.7% (116)	40.5% (128)
5511	22.8% (72)	39.9% (126)	37.3% (118)
6040	22.8% (72)	40.2% (127)	37.0% (117)
3707	22.5% (71)	42.1% (133)	35.4% (112)
7377	22.5% (71)	35.8% (113)	41.8% (132)
7557	22.2% (70)	43.0% (136)	34.8% (110)
5715	21.8% (69)	40.2% (127)	38.0% (120)
0331	21.5% (68)	45.3% (143)	33.2% (105)
3777	21.5% (68)	35.4% (112)	43.0% (136)
5765	21.2% (67)	42.4% (134)	36.4% (115)
4611	20.6% (65)	45.9% (145)	33.5% (106)
4776	19.9% (63)	40.5% (128)	39.6% (125)
5735	19.6% (62)	40.2% (127)	40.2% (127)
2567	18.7% (59)	40.8% (129)	40.5% (128)
4523	18.7% (59)	51.3% (162)	30.1% (95)
4761	18.7% (59)	48.7% (154)	32.6% (103)
5137	18.7% (59)	38.3% (121)	43.0% (136)
6045	18.4% (58)	53.2% (168)	28.5% (90)
7777	15.2% (48)	42.4% (134)	42.4% (134)
5425	14.6% (46)	53.5% (169)	32.0% (110)
6737	13.0% (41)	40.5% (128)	46.5% (147)
7737	12.7% (40)	51.6% (163)	35.8% (113)
Q-301	11.7% (37)	40.5% (128)	47.8% (151)
H-148	8.2% (26)	41.8% (132)	50.0% (158)
S-016	6.6% (21)	29.1% (92)	64.2% (203)
J-462	3.5% (11)	34.2% (108)	62.3% (197)
Y-035	3.2% (10)	43.4% (137)	53.5% (169)
Y-069	3.2% (10)	22.5% (71)	74.4% (235)

[1]Resistant was defined as any accession that responded with a 0 to 1.5 transformed Infection type (IT)
[2]Intermediate reactions was defined as any accession that responded with a 2 to 2.5 IT
[3]Susceptible reactions was defined as any accession that responded with a 3 to 4 IT
[4] Class types indicate the approximate shape of each of the distributions, whether the frequency scores were concentrated at 0 IT (Class 1), were evenly split between 0 IT and a normal distribution between 1.5 and 4 IT (Class 2), or were dominated by a normal distribution between 1.5 and 4 IT (Class

Table 5. **Correlations of transformed IT exhibited by the WBDC accessions with geographical and environmental factors**.

Table of correlations	Precipitation per year	Yearly Max Temperature	Yearly Min Temperature	Aridity
0004	0.09	**-0.21	*-0.16	0.1
0020	0.05	*-0.17	*-0.15	0.08
0023	0.06	*-0.16	*-0.13	0.08
0061	0	-0.1	-0.07	0.01
0235	0.03	*-0.18	*-0.14	0.04
0323	0	**-0.19	*-0.18	0.03
0331	0.06	**-0.22	*-0.16	0.09
0574	-0.02	*-0.18	*-0.17	0.02
1002	0.06	*-0.17	*-0.14	0.08
1044	-0.01	**-0.32	**-0.28	0.05
1377	-0.01	**-0.19	**-0.19	0.02
1541	0.05	*-0.16	-0.09	0.06
2567	0.03	**-0.2	*-0.18	0.07
3707	0.02	**-0.2	*-0.17	0.06
3777	-0.01	-0.11	-0.09	0.02
4404	0.02	*-0.18	*-0.18	0.03
4523	-0.09	-0.1	-0.07	-0.07
4611	0.03	*-0.13	-0.08	0.04
4761	-0.04	*-0.16	*-0.17	-0.02
4776	-0.11	*-0.13	*-0.16	-0.09
5137	-0.04	*-0.17	*-0.16	0
5425	0.02	*-0.17	*-0.14	0.03
5511	-0.01	*-0.15	-0.12	-0.01
5735	-0.05	*-0.16	*-0.14	-0.02
5765	-0.07	*-0.14	*-0.12	-0.05
5715	-0.02	-0.05	-0.02	-0.03
6000	-0.08	0.01	-0.01	-0.1
6040	-0.06	*-0.14	*-0.15	-0.04
6045	0	**-0.24	**-0.2	0.04
6737	-0.05	**-0.24	**-0.22	0
7377	*-0.13	-0.11	-0.11	-0.1
7557	0.04	-0.11	-0.07	0.04
7737	-0.07	*-0.14	*-0.15	-0.04
7777	-0.1	*-0.18	**-0.21	-0.06
H-148	*0.19	0.03	*0.13	*0.18
J-462	0.09	-0.1	-0.02	*0.15
Q-301	*0.16	-0.01	0.1	*0.15
S-016	*0.12	-0.04	-0.02	*0.12
Y-035	*0.19	-0.03	0.09	**0.19
Y-069	*0.13	-0.07	-0.01	*0.13
Aggregate	-0.01	*-0.18	*-0.15	0.01

Correlations for each of the univariate tests. * indicates .001<p<.05 and ** indicates p<.001

Table 6. **Number of accessions that are postulated to contain the respective gene(s) present in the Pallas near-isogenic lines.**

Mla1	Mla3	Mla6	Mla7	Mla9	Mla12	Mla13	Mlk1	MlLa	Mlg	Mlat	Ml(Ru2)
77	11	80	2	2	74	0	88	3	15	72	80

Figure 1. Map position of Wild Barley Diversity Collection accessions colored according to their aggregate transformed infection type value. The aggregate infection type value (sum of all transformed infection types) of accessions to all 40 powdery mildew isolates were calculated, colored coded according to their reaction spectra (green is most resistant and red is most susceptible) and geographically mapped to their original collection site.

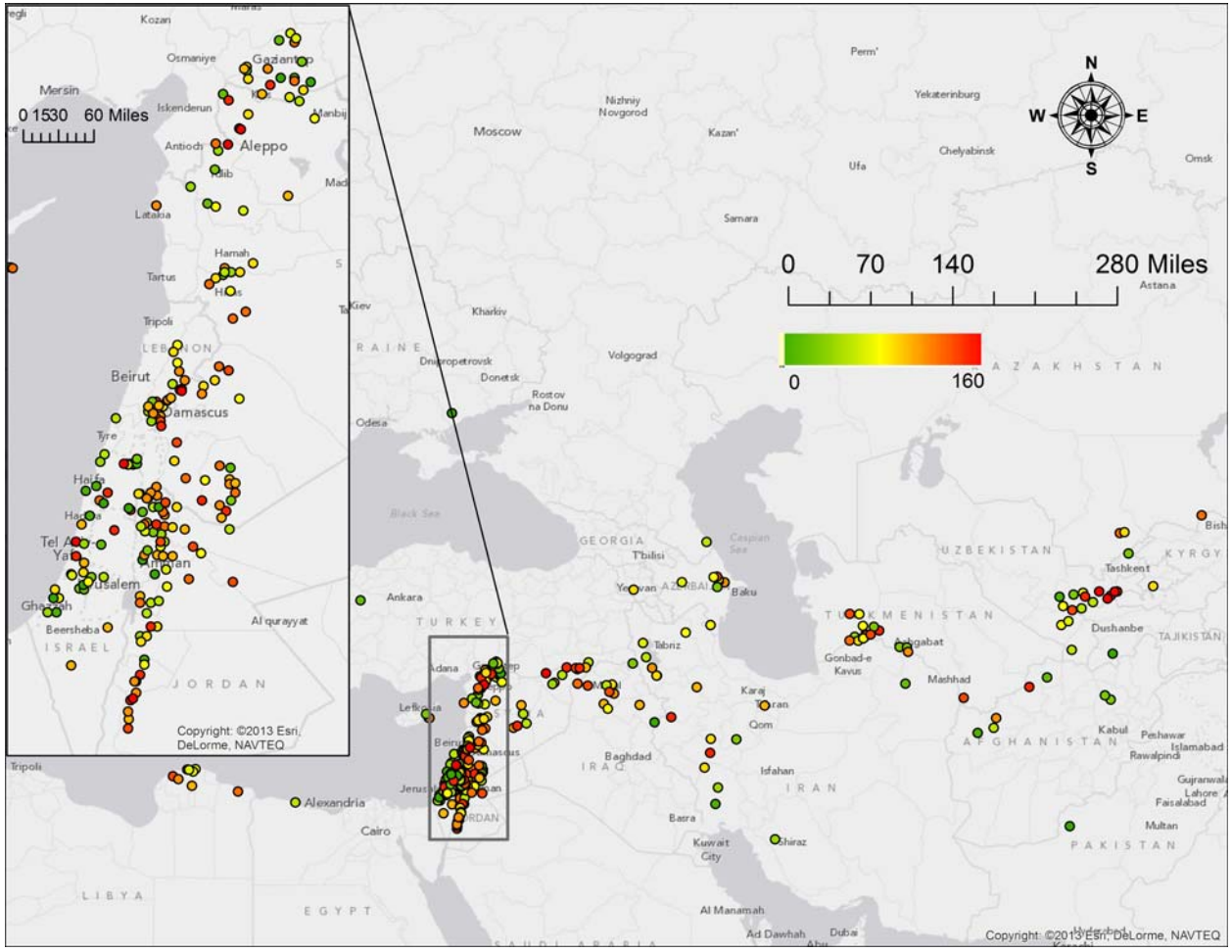
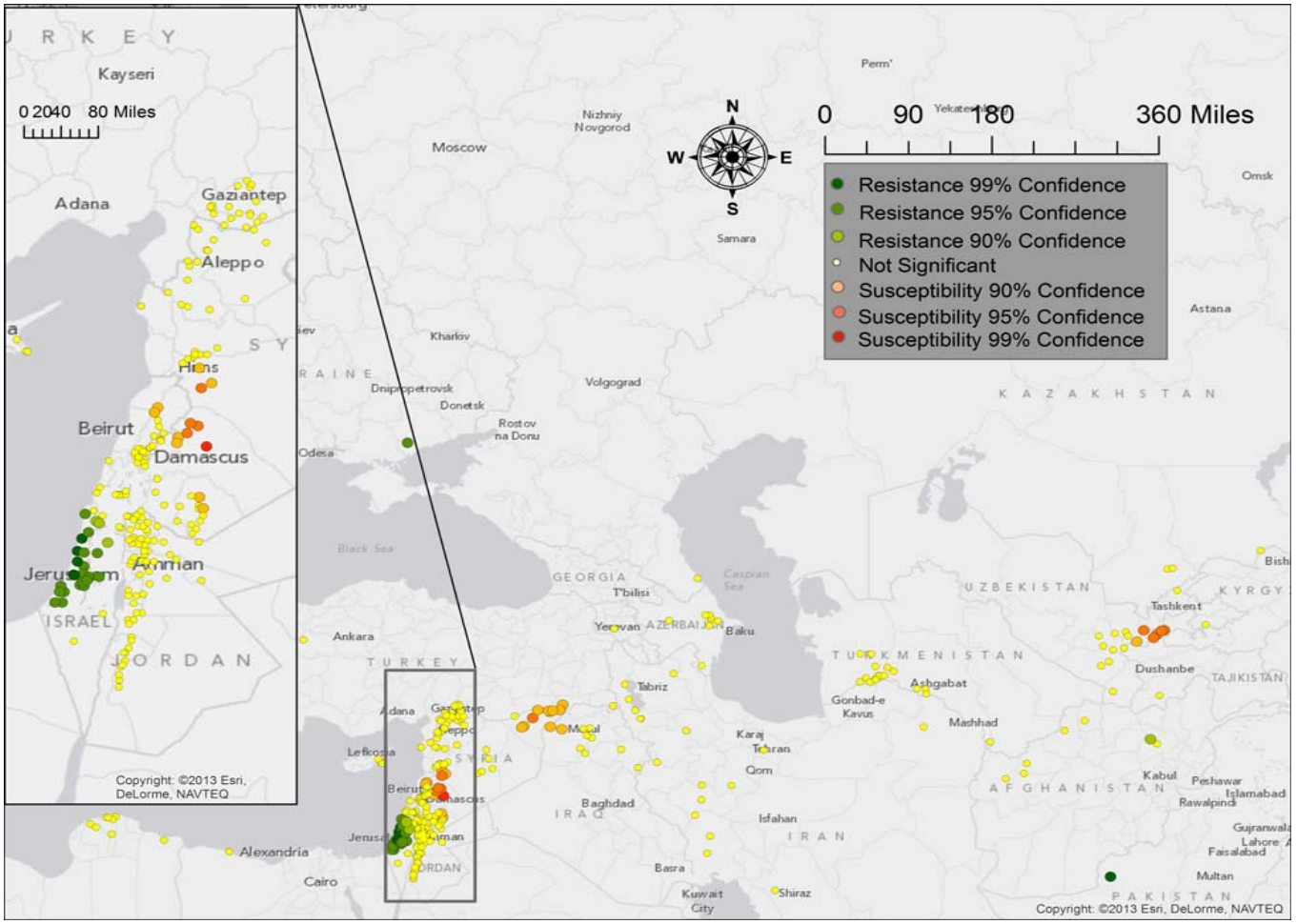


Figure 2. Hotspot analysis of resistance and susceptibility of Wild Barley Diversity Collection accessions based on the aggregate transformed infection type values to 40 powdery mildew isolates. Green spots of varying intensities indicate concentrations of resistance with different confidence levels whereas yellow to red spots indicate concentrations of susceptibility with different confidence levels. Yellow spots indicate that the accession was not implicated in a hotspot. Confidence levels indicate the Z-score for each datapoint, and represent the level of confidence in rejecting the null hypothesis that each point is not part of a cluster.



Chapter Three:

Genome-Wide Association Study for Resistance to Powdery Mildew in the Wild Barley Diversity Collection

Introduction

The powdery mildew pathogen (*Blumeria graminis* DC f. sp. *hordei* Ém. Marchal (*Bgh*)), of barley *(Hordeum vulgare* L.) is an important biotic constraint in many production areas of the world. In regions where barley is widely grown, powdery mildew causes more damage than any other single disease with yield losses ranging from 5-20%, and up to 40% in severe epidemic years (Chaure *et al.*, 2000; Dreiseitl, 2011b). A common method of control for this disease is the use of fungicides, but such additional inputs result in increased costs to producers and also possible problems with the development of fungicide-resistant strains in the pathogen population (Dekker, 1976; Staub, 1991; O'Brien, 1994; Fraaije *et al.*, 2002). The development and deployment of resistant cultivars is another means of combatting powdery mildew, but can suffer the fate of "boom and bust" cycles if the resistance is not broad-based (Jørgensen, 1983; Jahoor & Fischbeck, 1993; Dreiseitl, 2003, 2011a). Thus, it is essential that new cultivars be bred with broad-spectrum resistance genes.

Mla and *mlo* are two of the most effective and widely deployed powdery mildew resistance genes used in barley breeding. The *mlo* gene is a broad spectrum resistance gene located on chromosome 4H. It was originally identified in Ethiopian landraces, but was later reconfirmed in multiple forms from mutation studies (Büschges *et al.*, 1997). This gene has been highly effective in protecting European barley lines from powdery mildew losses for over 35 years. Unfortunately, in recent years, *mlo*-based resistance is slowly being eroded by adapted isolates of *Bgh* (Schwarzbach *et al.*, 2002). In contrast to *mlo*, *Mla* is a complex NBS-LRR locus on chromosome 1H that exhibits race-specific

resistance to *Bgh* (Jørgensen & Wolfe, 1994; Wei *et al.*, 1999). These genes together combine to form specific *Mla* alleles (eg. *Mla6, Mla12,* etc.). Many other powdery mildew resistance genes have been identified in barley (Jørgensen & Wolfe, 1994; Kintzios *et al.*, 1995; Dreiseitl *et al.*, 2007), but lack the extreme duplication and diversification that makes *Mla* such an effective source of resistance. *Mlo* on the other hand acts at a basal level to resist *Bgh,* and is one of the most effective sources of resistance in barley. Some of the other major resistance genes include *Mlat, Mlk, Mlnn, Mlra, MlGa,* and *Mlp* located on chromosome 1H (reviewed in Joergensen, 1994), *MlLa* on chromosome 2H (Hilbers *et al.*, 1992), *Mlg* on chromosome 4H (Görg *et al.*, 1993; Baker *et al.*, 1997), and *Mlh* on chromosome 6 (Jørgensen & Wolfe, 1994).

Promising sources for powdery mildew resistance in barley include landraces (*Hordeum vulgare* L. subsp. *vulgare*) and wild barley (*Hordeum vulgare* subsp. *spontaneum).* Indeed, previous screening of landrace (Jahoor & Fischbeck, 1987a; Czembor & Johnston, 1999; Czembor, 2000a, 2001; Czembor & Czembor, 2002; Dreiseitl & Bockelman, 2003; Fetch *et al.*, 2003), wild barley (Jahoor *et al.*, 1989; Spaner *et al.*, 1998; Dreiseitl *et al.*, 2007; Dreiseitl & Platz, 2012), and *Hordeum bulbosum* (Xu & Kasha, 1992; Pickering & Hill, 1995) accessions identified novel sources of resistance to powdery mildew. Another possible source of powdery mildew resistance is the Wild Barley Diversity Collection (WBDC), a core collection of wild barley accessions drawn together for assessing diversity in this important subspecies of barley. The WBDC is composed of 318 geo-referenced wild barley accessions obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA) and was assembled to

sample as much of the range and climate conditions of wild barley as possible. It was screened for resistance against 40 *Bgh* isolates and found to contain a wide variety of resistance spectra among individual accessions (Ames et al. Chapter 2). To enable the efficient use of this valuable germplasm resource for barley improvement, the chromosomal location of *Bgh* resistance loci is needed.

The traditional approach to genetic mapping has been to associate genotype and phenotype data from segregating populations derived from bi-parental crosses. However, this process is time-consuming due to the need to create biparental populations with the appropriate parents. Recently, genome-wide association studies (GWAS) have become popular for mapping numerous traits in plants (Visscher *et al.*, 2012). GWAS is based on the association between the genotypes of a collection of germplasm and their phenotypes after accounting for population structure (Myles *et al.*, 2009; Painter *et al.*, 2011). GWAS in barley has been used successfully to map loci controlling agronomic traits (von Zitzewitz *et al.*, 2011; Wang *et al.*, 2012; Pasam *et al.*, 2012; Berger *et al.*, 2013; Pauli *et al.*, 2014; Muñoz-Amatriaín *et al.*, 2014), disease resistance (Steffenson *et al.*, 2007; Massman *et al.*, 2010; Roy *et al.*, 2010), food quality (Mohammadi *et al.*, 2014), morphological traits (Cockram *et al.*, 2010; Ramsay *et al.*, 2011; Tondelli *et al.*, 2013), and frost resistance (Visioni *et al.*, 2013).

The overall goal of this study was to conduct GWAS in the WBDC for resistance to forty races of *Bgh*. Our two specific objectives were to: (1) identify novel and known loci for powdery mildew resistance in the WBDC, (2) identify novel powdery mildew

resistance loci and (2) validate loci found previously associated with powdery mildew resistance.

Materials and Methods

Plant materials and phenotyping

Details on the attributes and handling of the pathogen cultures, the testing procedures used for inoculating and scoring the reactions of the WBDC, and also techniques for data analysis were previously reported in Ames et al. (Chapter 2).

Genotyping

Three WBDC seeds were grown in a 50/50 mix of steam sterilized native soil and sunshine MVP potting mix (Sungro Horticulture Distributor's Inc.). Plants were placed in the cold room (4° C) for 7 days to break dormancy. Seedlings were grown in greenhouse 485-7, Plant Growth Facilities East, St. Paul until about 14 days after plants were removed from the cold room. The youngest leaves were removed and placed in a 96 well plate and stored at -80° C and subsequently lyophilized. DNA extraction was performed on a single plant, using a QIAGEN Biosprint 96 DNA Plant Kit (QIAGEN; cat no: 951558). A single sample (WBDC131) was processed using the DNEasy Plant Mini Kit (QIAGEN, cat no. 69106).

DNA samples were processed for DArT-seg by a series of digestion/ligation reactions as previously reported (Kilian *et al.*, 2012), but replacing a single *PstI*-compatible adapter with two different adapters corresponding to two different Restriction Enzyme overhangs. The *PstI*-compatible adapter was designed to include the Illumina flowcell attachment sequence, sequencing primer sequence, and a "staggered", varying length barcode region similar to the sequence reported by (Elshire *et al.*, 2011). The

reverse adapter here contained the flowcell attachment region and the *HpaII*-compatible overhang sequence. Only "mixed fragments" (*PstI-HpaII*) were effectively amplified in 30 rounds of PCR using the following reaction conditions: 94°C for 1 min, followed by 29 cycles of 94°C for 20 sec, ramp to 58°C, 58°C for 30 sec, ramp to 72°C, 72°C for 45 sec. Amplicons were then held at 72°C for 7 min and then at 10°C at the end of amplification.

After PCR, equimolar amounts of amplification products from each sample of the 96-well micro-titer plate were bulked and applied to c-Bot (Illumina, San Diego, California) bridge PCR followed by sequencing on an Illumina Hiseq2000. The sequencing (single read) was run for 77 cycles.

Sequences generated from each lane were processed using proprietary DArT analytical pipelines. In the primary pipeline, the fastq files were first processed to filter away poor quality sequences, applying more stringent selection criteria to the barcode region compared to the rest of the sequence. In that way the assignments of the sequences to specific samples carried in the "barcode split" step were very reliable. Approximately 2,000,000 sequences per barcode/sample were identified and used in marker calling. Finally, identical sequences were collapsed into "fastqcall files." These files were used in the secondary pipeline for DArT PL's proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14).

SNP and DaRT markers were also used to genotype the WBDC (Sansaloni *et al.*, 2011). BOPA1 and BOPA2 (3,072 SNPs; Close *et al.*, 2009) SNP alleles were assessed

previously on the WBDC (Roy *et al.*, 2010). DArT markers (Steffenson *et al.*, 2007) were also genotyped on the WBDC.

Quality control

Quality control on the initial dataset of SNPs, DaRT, and DaRT-Seq markers was conducted in the following fashion: all heterozygous and monomorphic markers were removed, as were markers with >10% missing calls. After this filtering protocol 2,597 SNP, 545 DaRT, and 8,616 DaRT-Seq markers remained. Two of the 318 WBDC accessions were removed due to >10% missing data. Two more accessions were removed due to strong evidence that they were admixed largely with domesticated barley. To calculate kinship, population structure and LD, a 1% minor allele frequency threshold was imposed for all marker types resulting in 2,359 SNPs, 536 DaRT and 7,613 DaRT-Seq markers.

Population structure analysis

To estimate the number of subpopulations in the WBDC, STRUCTURE v2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2003; Falush *et al.*, 2007) and Principal Component Analysis (PCA) were used. A burn-in time of 10,000 with 10,000 reps, 10 times for each predicted K was used for the initial prediction with 2,359 SNP markers. To identify a stable number of subpopulations, Delta K was calculated (Evanno *et al.*, 2005). This population structure output was used to assess the number of stable subpopulations,

ANOVA analysis, and as the Q covariate for the GWAS study for the SNP markers. It was also used for map assignments, though each accession was colored according to the majority subpopulation. The same conditions (10,000 burn-in with 10,000 reps, 10 times for each K) were used for each of the DaRT and DaRT-Seq markers, but results were only used as Q covariates for the respective marker sets.

Mixed Linear Model analysis

GWAS was conducted using TASSEL v 3.0.148 (Bradbury *et al.*, 2007). Each of the marker sets (DaRT, DaRT-Seq, and SNP) were analyzed separately. The kinship matrix used was generated in TASSEL from a 1% minor allele filter screened for SNP, DaRT, and DaRT-Seq markers resulting in 2,359, 536 and 7,613 markers, respectively. STRUCTURE results were used as Q covariates, with the kinship matrix used as the K portion of the analysis. STRUCTURE Q matrices were calculated for their respective marker sets. Mixed Linear Model analysis uses population structure (Pritchard *et al.*, 2000; Zhao *et al.*, 2007) as a fixed effect, with kinship used as a variance-covariance structure for the random effect of individuals (Zhang *et al.*, 2010). A threshold for significance was also calculated for models using a Q-value correction method for each of the *Bgh* pathotype association studies to account for multiple testing error (Storey, 2002; Storey & Tibshirani, 2003; Storey *et al.*, 2004).

Linkage analysis

2,359 SNP markers (those markers over .01 MAF) were imported into the program Haploview (Barrett *et al.*, 2005; Barrett, 2009) to describe the level of LD within the population. Output from TASSEL version 3.0.148 (Bradbury *et al.*, 2007) was also used to examine the level of LD decay on each of the chromosomes. We also measured the level of LD with the DaRT-Seq marker set. For the DaRT-Seq markers, we randomly chose one marker per site (as many of the markers measure the state at a site multiple times), and filtered out only markers with known positions leaving us with 2,870 DaRT-Seq markers to evaluate LD.

Results

WBDC population structure

Population structure of the WBDC was assessed using STRUCTURE and Principal Component Analysis (PCA) on 2,359 SNP markers. Calculations for 1-10 subpopulations were examined, with seven subpopulations predicted as the most stable number of subpopulations (Figure 1A-1B). PCA eigenvectors were plotted along the X-Y-Z axis, and accessions were colored according to their respective subpopulation. Forty-four heavily admixed accessions (those that were not postulated to at least have 50% of their genomic content belong to at least a single accession) were omitted from the figure (Figure 1A-1B). For ease of reference for each successive figure referencing the subpopulations, we retained the color in this figure. We also labeled the subpopulations by their region. The PCA confirms the separation of each of the STRUCTURE subpopulations. Geographical mapping of each subpopulation shows separation, with some admixture in small areas (e.g., Israel) (Figure 1).

Phenotypic data

In a previous study, we obtained infection type (IT) data for each WBDC accession for 40 different *Bgh* isolates (Ames et al., Chapter 2). We examined the mean ITs for accessions in each of the subpopulations to each pathotype using an ANOVA procedure and found statistically significant differences among 34 of the 40 isolates, indicating that the subpopulations exhibit different reactions to powdery mildew (Figure 2, Table 1). This suggests that the sub-populations were effective in separating accessions

with different IT means. While some populations were larger than others (the smallest being the Transcaucaus subpopulation (sp 1) with only 10 accessions), the rest of the populations were more evenly distributed, with the populations containing 66, 67, 46, 42, 35, and 48 accessions, respectively (sp 2, 3, 4, 5, 6, and 7). The population which exhibited the most mean resistance (North Africa/West Israel) had 66 accessions, while the population that showed the least overall resistance had only 35 accessions. Subpopulation 3, despite having the most members, showed only moderate mean resistance in comparison to the rest of the subpopulations. The separation was less notable in virulent isolates such as H-148, J-462, Q-301, S-016, Y-035, and Y-069 which had much higher average IT scores than the rest of the isolates tested (Table 1). In addition, the geographic grouping and IT differences among subpopulations, indicates that there are broad geographic areas where resistance to many isolates is more likely to be found. In particular, the subpopulation for North Africa/western Israel had a lower mean resistant IT score than any other subpopulation across 33/40 isolates tested. The Eastern Israel subpopulation (the East Jordan-Israel population in Figure 2, 3) had the highest mean IT score across a majority of the isolates tested (25/40). Other than these two examples, no strong overall patterns were observed with respect to the reaction of subpopulations to all isolates. No significant differences were observed among the subpopulations to the two most highly virulent isolates, S-016 and Y-069. The rest of the virulent isolates (H-148, Y-035, Q-301, J-462) showed no sub-populations that were consistently resistant, but did show significant separation among the subpopulations.

Association mapping of QTL for powdery mildew resistance

To identify loci for *Bgh* resistance, we analyzed the genotypic data of 2,597 SNPs, 545 DaRT and 8,616 DaRT-Seq markers together with the phenotypic IT data of the WBDC in a GWAS. GWAS was conducted using TASSEL v 3.0.148. The significant markers were then corrected by the QVALUE program (Storey & Tibshirani, 2003) to account for multiple testing errors. Nineteen markers were found significantly associated with *Bgh* resistance after correction ($p<0.05$). Two markers (bpb-0789 and 12_10959) were found associated with resistance to more than one isolate. One pair of markers were within 5 cM of each other. Markers associated with resistance were found on all chromosomes, except chromosome 1H (Table 2, Figure 4). Loci that confer resistance to isolates Y-069, Q-301, 0331, 5137, 1541, 6045, 7557, 0323, 5715, H-148, and S-016 were all found.

Based on the number of accessions that exhibited resistance to the 40 isolates, the most widely virulent races used in this study were Y-069, Y-035, J-462, Q-301, H-148, and S-016 (Ames, Chapter 2). Few of the WBDC accessions (3.16%, 3.16%, 3.48%, 11.7%, 8.22%, and 6.64% of all accessions, respectively) exhibited complete resistance to these isolates. Surprisingly, many loci conferred resistance to these widely virulent races (Figure 4, Table 2). Of all the significantly associated markers found, a majority of them (12/19) were associated with resistance to a highly virulent pathotype. Markers Bgh-qtl-2H-100006327|F|0, Bgh-qtl-2H-100004506|F|0, Bgh-qtl-2H-100000555|F|0, Bgh-qtl-3H-100006760|F|0, Bgh-qtl-5H-100004344|F|0, Bgh-qtl-U-100003758|F|0, Bgh-qtl-2H-100000522|F|0, Bgh-qtl-4H-12_31258, Bgh-qtl-6H-100001292|F|0, Bgh-qtl-7H-

12_10959, Bgh-qtl-U-100020328|F|0, Bgh-qtl-7H-100001459|F|0 all conferred resistance to extremely virulent isolates (Chapter 2, Table 4).

Loci conferring resistance to multiple isolates

Three of the loci exhibited resistance to two isolates. The QTL *Bgh-qtl-3H-bPb-0789* (associated with resistance to isolates 5137 and 1541) and *Bgh-qtl-7H-12_10959* (resistance to isolates Q-301 and H-148) conferred resistance to multiple isolates using a single marker in both locations (3H at 149 cM and 7H at 52.5 cM respectively). Bgh-qtl-4H-100001039|F|0 and Bgh-qtl-4H-100004309|F|0 were resistant to different isolates (isolates 7557 and 0331), and were within 5cM of each other. The virulence phenotypes of these isolates (Chapter 2, Table 1) was examined to see if there is a resistance gene in the Pallas lines that would explain these common sites. Isolates 5137 and 1541 only share common avirulence on *Mla*3 and *Mla*9. As the locus identified is on chromosome 4H, and *Mla* is on chromosome 1H, *Mla* is unlikely to be involved in this common resistance. For the Q-301 and H-148 pathotype pair, the common avirulence pattern was unable to be studied, as Q-301 was unavailable for evaluation on the Pallas isolines. H-148 was only avirulent for *Mla3, Mla7,* and *Mla13*. Again, the locus was on 7H, and so *Mla* is unlikely to be responsible for this common resistance. Isolates 7557 and 0331 shared no common avirulence genes for any resistance gene in the Pallas NILs. Interestingly, these markers are close to *Mlg*, but neither isolate is avirulent with respect to *Mlg*.

Linkage disequilibrium

Previously (Ames, Chapter 2), we postulated that 93 of the WBDC accessions contain an allele at the *Mla* locus. However, we did not detect any markers that were associated with *Bgh* resistance mapping to the *Mla* region (approximately 38 cM) on chromosome 1H. This could be the result of low linkage disequilibrium (LD) and the limited number of the markers used for genotyping the WBDC. To assess the LD across the genome and specifically at the *Mla* region of the genome, we imported all markers into Haploview and Tassel (Barrett *et al.*, 2005; Barrett, 2009) (Figure 5, Figure 6). Low levels of LD were observed across each chromosome, with a 25 marker sliding window analysis indicating an average R^2 of 0.022 across all seven chromosomes. We examined LD near the *Mla* locus on chromosome 1H and observed low LD within the region among adjacent loci, indicating that the marker coverage was insufficient to detect *Mla*. In addition to this, LD decay across the chromosome is rapid enough (average R-squared values fall to 0.048 for markers that are 0.5 cM away from each other, and fall below 0.01 for markers that are between 5.5 and 6 cM away from each other) to suggest that our coverage may not be sufficient for detecting all resistance loci. Previous surveys of wild barley accessions have often detected *Mla* alleles (Jahoor & Fischbeck, 1987c, 1993; von Korff *et al.*, 2005; Řepková *et al.*, 2006; Yun *et al.*, 2006; Dreiseitl *et al.*, 2007); however, it is possible that certain *Mla* alleles are not present in high enough frequencies to be detected by GWAS. In our previous postulation of genes within the collection (Ames Chapter 2), we postulated moderate frequencies of *Mla*1, *Mla*6, and *Mla*12 (77, 80, and 74 accessions present, respectively). We would expect the allele frequency of

Mla within the WBDC would be high enough to be detected by GWAS. The inability to detect *Mla* here is more likely a problem with the limited marker coverage of the genome.

Discussion

Powdery mildew is a highly damaging, greatly mobile, and rapidly-evolving pathogen that can cause between heavy yield losses in barley under epidemic conditions. Current gene deployment strategies include the use of *mlo* and *Mla*. However, breeding efforts must constantly find new alleles for the *Mla* gene to keep it effective. The *mlo* gene, while conferring broad-based resistance and having been effective for the last 35 years, is not at all used in winter barley due to concerns that its use in both spring and winter type barley would put undue selection pressure on the pathogen to overcome the resistance gene. Due to the diversity of powdery mildew races and powdery mildew's dynamic ability to overcome resistance genes quickly, with the exception of *mlo*, new genes are constantly needed in places where barley is grown and consequently where it is most economically important. The WBDC is a diverse and exotic germplasm collection that contains a rich resource of accessions with powdery mildew resistance (Ames, Chapter 2). An association mapping approach was used to mine the WBDC for novel loci and alleles and identified both previously identified as well as putatively novel disease resistance loci.

Population substructure and geographical location is associated with differences in resistance

Previous work with the WBDC indicated ten and eight subpopulations using DaRT markers and SNP markers (though with different MAF thresholds) (Steffenson *et al.*, 2007; Roy *et al.*, 2010). Recent work suggests five (using fewer accessions from the WBDC (Russell *et al.*, 2014)) and six sub-populations (using stricter MAF thresholds and

alternative methods than those used here (Fang *et al.*, 2014)). Given these different analyses, our assignment of seven sub-populations seems rational in light of the geographic grouping and PCA corroboration.

When the mean infection type scores for each pathotype within each sub-population were examined, significant differences were observed among the latter (Table 1, Figure 2). Considering also that these subpopulations were from geographically distinct areas, there were geographic concentrations of where powdery mildew resistance was found. Multiple clusters of distinct genetic populations were observed across Israel, consistent with previous examinations and observations that this region contains a high level of genotypic diversity for wild barley (Fig. 3). These clusters seem to occupy the same geographic space in the densely sampled sections of Israel and Jordan, suggesting that there are multiple distinct genotypic populations in the same environment. While other regions are less dense with respect to sampled accessions, they still remain relatively uniform, aside from a few incidental accessions. This high level of diversity present in accessions from Israel and Jordan compared to those from other regions has also previously been noted (Nevo *et al.*, 1979; Fu & Horbach, 2012). This spatially close, but diverse group of admixed barley populations may have contributed to the cluster of resistance found in the region. The separation of mean ITs across the sub-population groupings also means that the Q-matrix was effective in mitigating population structure effects. This effect is also confirmed in our previous findings of a hotspot in western Israel (Ames, Chapter 2).

This cluster of resistance and this collection in general may be a target in the future for the Focused Identification of Germplasm Strategy (FIGS). It has been used to locate accessions within large germplasm collections that may be resistant to pathogens based on their environmental profiles (Mackay & Street, 2004; Bonman *et al.*, 2005; El Bouhssini *et al.*, 2011). Using the pathotype scores, as well as collecting more complete information on the environment of collection, we can further identify accessions within ICARDA (or other genebanks) that are not part of the WBDC, or guide further collection efforts in areas that fit the environmental profile of resistant accessions.

Powdery mildew resistance QTL identified

Of the significantly associated markers detected in this study, a majority (11) of them are in chromosomal locations that have previously been reported in other population analyses (Figure 4) with the percentage of resistance explained ranging between 5.1% and 8.2%. Two markers, Bgh-qtl-4H-100001039|F|0 (7557) and Bgh-qtl-4H-100004309|F|0 (0331) on chromosome 4H were found in the same region as the *Mlg* locus (Kurth *et al.*, 2001). A marker on chromosome 2H at 146 cM (Bgh-qtl-2H-100000522|F|0, Q-301) was also found close to the previously reported position for *MlLa* (Giese *et al.*, 1993) and QTL *Rbgq9* (Aghnoum *et al.*, 2010). A marker on chromosome 3H (Bgh-qtl-3H-bPb-0789, 5137/1541) was found in the same region as an *mlo* mediated resistant locus (Zierold *et al.*, 2005; Aghnoum *et al.*, 2010). Eight loci (2H at 146 cM [Q-301], all loci on 3H [0331, Y-069, 5137, 1541], 5H at 47 cM [Y-069], 6H at 104 cM [Q-301], and both loci on 7H [Q-301/H-148, Q-301]) were found in coincident locations with previously detected resistance loci (Aghnoum *et al.*, 2010), including *Rbgq9* (which

also corresponds to *MlLa*), *Rbgq2a*, *Rbgq11*, *Rbgq14*, *Rbgq19*, and *Rbgq22*. In addition, we detected marker Bgh-qtl-2H-100000555|F|0 (Y-069) in a region of chromosome 2H that was previously shown to contain a powdery mildew resistance QTL lang1031QPm.S42-2H.a (von Korff *et al.*, 2005).

We found six novel loci; two on 2H (107cM and 129 cM, both resistant to Y-069), two on 4H (11cM and 86 cM, resistant to 6045 and Q-301, respectively), one on 5H (167 cM, resistant to 0323), and one on 6H (17.8 cM, resistant to 5715). The markers explained between 5.9% and 7.2% of the variation. We did not identify a novel locus that exhibited resistance to more than one pathotype. Two additional unmapped markers (Bgh-qtl-U-100003758|F|0 and Bgh-qtl-U-100020328|F|0) were associated with resistance to isolates Y-069 and S-015, respectively. As these are unmapped it is not known if these are novel loci.

Bgh-qtl-7H-12_10959, Bgh-qtl-3H-bPb-0789, and the Bgh-qtl-4H-100001039|F|0; Bgh-qtl-4H-100004309|F|0 pair) confer resistance to more than one pathotype (i.e. 5137 and 1541). One of these markers (Bgh-qtl-3H-bPb-0789) is in the same location as an *mlo*-mediated resistance gene (WBE218) (Zierold *et al.*, 2005).

Previously, we postulated in the WBDC the presence of 12 powdery mildew resistance genes (*Mla1*, *Mla3*, *Mla6*, *Mla7*, *Mla9*, *Mla12*, *Mla13*, *Mlk1*, *MlLa Mlg*, *Mlat*, *Ml(Ru2)*) based on the comparative infection responses observed for Pallas NILs to 40 isolates of *Bgh* (Ames, Chapter 2). In this analysis, no marker-trait associations were found near *Mla* within the designated threshold. This is likely due to a low level of LD,

and a lack of SNP markers that map specifically to the *Mla* region (Figure 5, Figure 6). We also examined LD based on the DaRT-Seq markers, and found similar results (results not shown). Since none of the markers mapped close to the *Mla* locus, it is possible that the lack of marker-trait association is due to low levels of LD in the *Mla* region. These results and analysis of LD decay within the region confirm the rapid decay of LD, and a low level of LD between markers in the region. It is also possible that the diverse nature of our panel and the multi-allelic nature of the *Mla* gene, contributed to an allele frequency too low to be detected in a GWAS scan. Additionally, this null result may also demonstrate a weakness inherent in GWAS studies, the presence of many genes of large effect may effectively mask each other from detection. Many markers for resistance to highly virulent isolates were found, but fewer markers associated with resistance were found for highly avirulent isolates.

Of the remainder of genes that were postulated (*Mlk1, MlLa, Mlg, Mlat, Ml(Ru2)*) in the GWAS presented here only *Mlg* was detected. This result was surprising given that *Mlg* was postulated to be present in only 15 of the accessions, whereas *Mlk1, Mlat, and Ml(Ru2)* (all located on chromosome 1H) were postulated at in much higher frequencies (88, 72, and 80 accessions, respectively).

Utility of the WBDC for breeding powdery mildew resistance

Previous work has shown that the WBDC contains a rich resource of powdery mildew resistances (Ames, Chapter 2). The marker-trait associations described in this paper provide the opportunity to more effectively and efficiently use these loci in

breeding programs. Given the low LD within the WDBC and the potentially high value of resistance genes, the germplasm would greatly benefit from the use of additional genotyping such as the Infinium iSelect 9k SNP-chip (Kilian & Graner, 2012), genotyping-by-sequencing (Poland & Rife, 2012), exome capture sequencing (Choi *et al.*, 2009) or possibly full genome sequencing as sequencing costs continue to plummet (Wheeler *et al.*, 2008; Mills *et al.*, 2011). Considering that the WBDC has previously been screened for resistance to many other pathogens and for many other traits, the payoff from more extensive genotyping would be great not only for powdery mildew, but for other diseases as well.

To exploit wild barley most effectively in breeding, one must strongly select against deleterious alleles, i.e. those introduced by linkage drag. Previous QTL evaluations of wild barley collections have involved an advanced backcross (AB) scheme (Pillen *et al.*, 2003; Yun *et al.*, 2006; Li *et al.*, 2006; von Korff *et al.*, 2006; Gyenis *et al.*, 2007; Schmalenbach *et al.*, 2009). Advanced backcross schemes consist of the wild relatives of interest being back-crossed to the cultivated parent. Several AB collections (Yun *et al.*, 2005; von Korff *et al.*, 2005; Schmalenbach *et al.*, 2008) have been evaluated for powdery mildew resistance. Yun and colleagues (2005) identified two loci corresponding to *Mlg* and *Mla* loci. Van Korff and colleagues (2005) found nine QTL, including *Mla, Ppd-H1, MlLa, denso, Mlg, Mlj,* and *Mlf*, with two novel loci that had no candidate genes. Schmalenbach and colleagues (2008) identified seven QTL: four novel QTL, with three previously identified genes, including *Mla, Mlg,* and *Mlf.* There may besome value in re-evaluating these collections with new isolates in the future. In

addition, an AB population has been developed from 25 of the WBDC accessions that may prove useful in further validating powdery mildew resistance, or in providing a bridge for introgression into cultivated varieties. We evaluated three segregating families (of ~30 accessions each) for resistance to a race of the powdery mildew pathogen found in Minnesota that was similar to pathotype 1377 (data not shown). Significant QTL were not found either through GWAS, or through the use of a Haley-Knott regression using the map positions (results not shown). This was somewhat surprising, given the fact that each of the families showed overall moderate amounts of resistance, and that no individual was as susceptible as the susceptible parent. The most likely explanation for this failure is both a small family size, and that many loci contribute to the resistance, as suggested by the phenotypic distributions reported previously (Chapter 2).

Table 1. **Average powdery mildew infection type score for 316 Wild Barley Diversity Collection accessions within each of seven sub-populations as identified by Structure.**

Bgh isolates	Sub-populations[1]						
	Transcaucas	North Africa, West Israel	North Israel, Lebanon, Syria	Southeast Israel, Iraq, Iran, Turkmenistan	Syria, Iraq, Iran	East Jordan-Israel, Southern Syria	Central Asia
0004	2.15	1.72	2.40	2.25	2.37	2.93	1.94
0020	2.40	1.74	2.42	2.21	2.36	2.60	1.89
0023	2.05	1.72	2.36	2.11	1.91	2.59	1.91
0061	1.90	1.86	2.25	2.40	2.27	2.44	1.90
0235	2.35	1.83	2.38	2.42	2.27	2.83	2.14
0323	2.45	1.89	2.31	2.37	2.50	2.90	2.32
0331	1.75	1.89	2.56	2.46	2.29	2.67	2.20
0574	2.40	1.83	2.36	2.35	2.20	2.51	2.17
1002	2.35	1.61	2.29	2.21	2.29	2.69	1.72
1044	2.40	1.04	1.59	1.90	1.26	2.13	1.98
1377	1.90	1.86	2.62	2.33	2.38	2.71	2.18
1541	2.35	1.69	2.37	2.32	2.13	2.46	1.87
2567	2.80	2.09	2.68	2.40	2.27	2.74	2.31
3707	2.55	1.91	2.49	2.48	2.17	2.63	2.00
3777	2.55	2.06	2.43	2.55	2.30	2.84	2.25
4404	1.55	1.56	2.21	2.32	2.56	2.81	2.24
4523	2.45	1.89	2.10	2.49	2.35	2.71	2.51
4611	2.65	2.03	2.43	2.51	2.32	2.74	2.29
4761	2.20	2.14	2.26	2.27	2.38	2.79	2.35
4776	2.90	1.85	2.16	2.39	2.56	2.84	2.66
5137	3.15	2.03	2.33	2.30	2.38	2.79	2.69
5425	2.60	1.96	2.38	2.44	2.33	2.53	2.68
5511	2.15	1.83	2.31	2.29	2.51	2.91	2.28
5735	2.75	2.14	2.43	2.24	2.39	2.80	2.57
5765	2.70	1.86	2.31	2.42	2.21	2.67	2.66
5715	2.15	2.01	2.21	2.47	2.50	2.70	2.44
6000	1.05	1.44	1.54	1.71	2.23	2.99	1.65
6040	2.30	1.85	2.35	2.26	2.39	2.79	2.49
6045	2.70	2.03	2.32	2.45	2.24	2.50	2.34
6737	3.25	2.26	2.48	2.51	2.31	2.93	2.95
7377	1.90	1.82	2.29	2.36	2.26	2.90	2.91
7557	2.10	2.05	2.42	2.35	2.50	2.71	2.24
7737	2.75	2.21	2.46	2.39	2.44	2.74	2.65
7777	2.70	2.08	2.50	2.53	2.55	2.77	2.78
H-148	2.50	2.79	2.86	2.64	2.41	2.67	2.42
J-462	3.50	2.90	2.88	2.79	2.62	2.70	2.82
O-301	2.10	2.80	2.81	2.53	2.35	2.54	2.45
S-016	3.00	2.80	3.08	2.76	2.91	2.76	2.87
Y-035	2.95	2.89	2.90	2.75	2.64	2.57	2.56
Y-069	3.05	3.11	3.12	2.87	2.95	3.19	3.06
Aggregate	97.45	81.02	96.63	95.79	94.25	108.70	94.29

[1]Sub-populations as defined by the STRUCTURE program.

Table 2. **List of molecular markers significantly associated with resistance to powdery mildew in the Wild Barley Diversity Collection.**

Marker Type	QTL	Pathotype	Marker Name	Chromosome	Position on chromosome in cM	P-value	Adjusted P-value	marker R^2(%)	MAF[2]	Possible previously identified coincident gene or QTL[3]
DaRT -Seq	*Bgh-qtl-2H-100000555|F|0*	Y-069	100000555|F|0	2H	18.91	9.70E-06	0.0194699	6.689335846	0.010101	lang1031QPm.S42-2H.a
DaRT -Seq	*Bgh-qtl-2H-100006327|F|0*	Y-069	100006327|F|0	2H	107.68	3.00E-05	0.03230625	6.287008665	0.0275862	Novel
DaRT -Seq	*Bgh-qtl-2H-100004506|F|0*	Y-069	100004506|F|0	2H	129.09	1.13E-05	0.0194699	7.179001477	0.0067797	Novel
DaRT -Seq	*Bgh-qtl-2H-100000522|F|0*	Q-301	100000522|F|0	2H	146.53	6.03E-06	3.33E-02	7.657430334	0.0068729	Rbgq9/MlLa
DaRT -Seq	*Bgh-qtl-3H-100002763|F|0*	0331	100002763|F|0	3H	64.87	1.74E-05	0.03833675	6.734406535	0.0207612	Rbgq2a
DaRT -Seq	*Bgh-qtl-3H-100006760|F|0*	Y-069	100006760|F|0	3H	104.39	1.06E-05	0.0194699	6.667188683	0.043771	Rbgq11
DaRT	*Bgh-qtl-3H-bPb-0789*	5137	bPb-0789	3H	148.82889	0.0001067	0.04472538	5.189022356	0.4228188	WBE218
DaRT	*Bgh-qtl-3H-bPb-0789*	1541	bPb-0789	3H	148.82889	6.029E-05	0.0328032	5.554191375	0.4228188	WBE218
SNP	*Bgh-qtl-4H-11_10319*	6045	11_10319	4H	11.0417626	3.266E-06	0.00849219	7.247526836	0.0127389	Novel
DaRT -Seq	*Bgh-qtl-4H-100001039|F|0*	7557	100001039|F|0	4H	54.32	4.96E-06	0.0427304	7.098975444	0.0392157	Mlg
DaRT -Seq	*Bgh-qtl-4H-100004309|F|0*	0331	100004309|F|0	4H	59.63	9.61E-07	0.004139508	8.245255116	0.009772	Mlg
SNP	*Bgh-qtl-4H-12_31258*	Q-301	12_31258	4H	83.7461108	2.262E-05	0.027685	5.93407702	0.066879	Novel
DaRT -Seq	*Bgh-qtl-5H-100004344|F|0*	Y-069	100004344|F|0	5H	47.01	2.46E-06	0.0113718	7.691890025	0.0033784	Rbgq14
SNP	*Bgh-qtl-5H-12_10257*	0323	12_10257	5H	167	6.255E-06	0.01623125	6.813975282	0.3407643	Novel
DaRT	*Bgh-qtl-6H-bPb-8473*	5715	bPb-8473	6H	17.85756	6.306E-05	0.0333549	6.698321753	0.1284047	Novel
DaRT -Seq	*Bgh-qtl-6H-100001292|F|0*	Q-301	100001292|F|0	6H	104.82	1.16E-05	0.03331133	6.776742949	0.0168919	Rbgq19
SNP	*Bgh-qtl-7H-12_10959*	Q-301	12_10959	7H	52.5171922	1.074E-05	0.026215	6.383666328	0.0127389	Rbgq22
SNP	*Bgh-qtl-7H-12_10959*	H-148	12_10959	7H	52.5171922	8.382E-07	0.002176286	8.191359011	0.0127389	Rbgq22
DaRT -Seq	*Bgh-qtl-7H-100001459|F|0*	Q-301	100001459|F|0	7H	62.18	8.63E-06	0.03331133	7.399880743	0.0588235	Rbgq22
DaRT -Seq	*Bgh-qtl-U-100003758|F|0*	Y-069	100003758|F|0	U	0	3.00E-05	0.03230625	6.597370321	0.026936	Novel?
DaRT -Seq	*Bgh-qtl-U-100020328|F|0*	S-016	100020328|F|0	U	0	4.46E-06	0.0384229	7.207875427	0.0292208	Novel?

[1]Pathotypes for which QTL was detected

[2]Minor allele frequency

[3]QTL indicates any previous found QTLs or genes that the site in proximity to, or if the site is novel

Figure 1A-1B. **Principal Component Analysis (PCA) plot of Wild Barley Diversity Collection accessions colored by STRUCTURE based on postulated subpopulation assignments** (shown above the PCA plot), where at least half of an accession belonged to a single subpopulation.

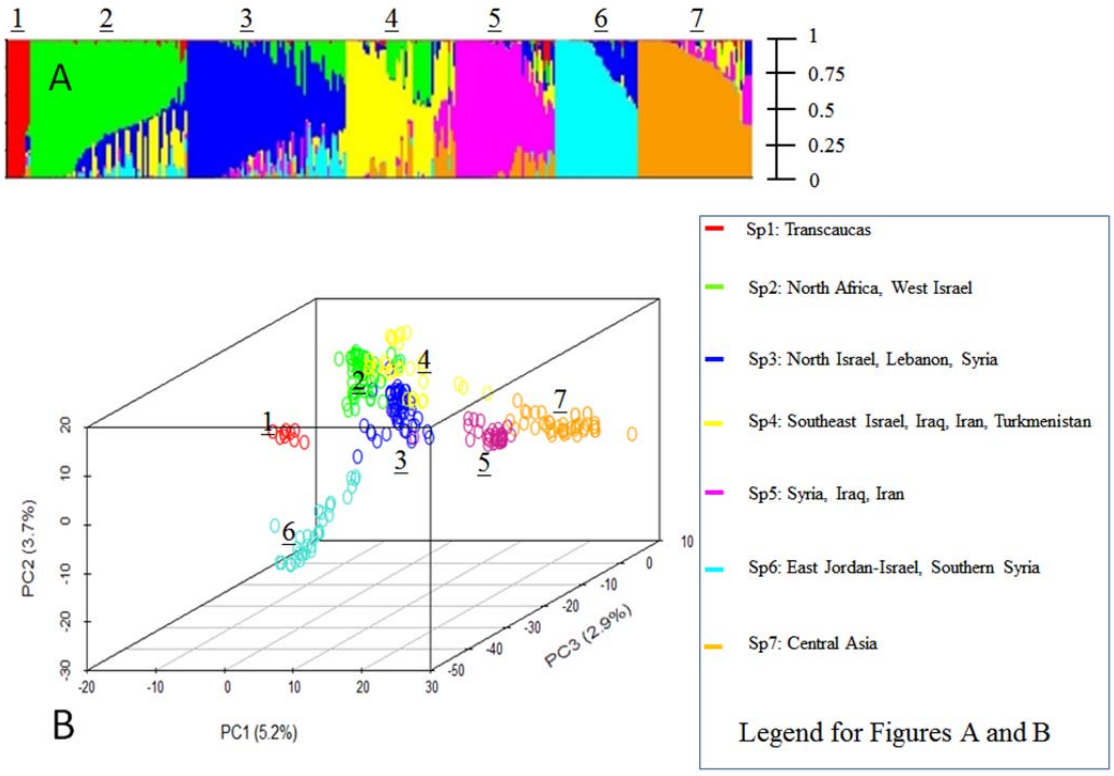


Figure 2. Mean powdery mildew infection types of each subpopulation of the Wild Barley Diversity Collection as defined by STRUCTURE in response to 40 isolates of *Blumeria graminis* f. sp. *hordei*. The separation of mean ITs among sub-populations indicates that they contain significantly different means of resistance.

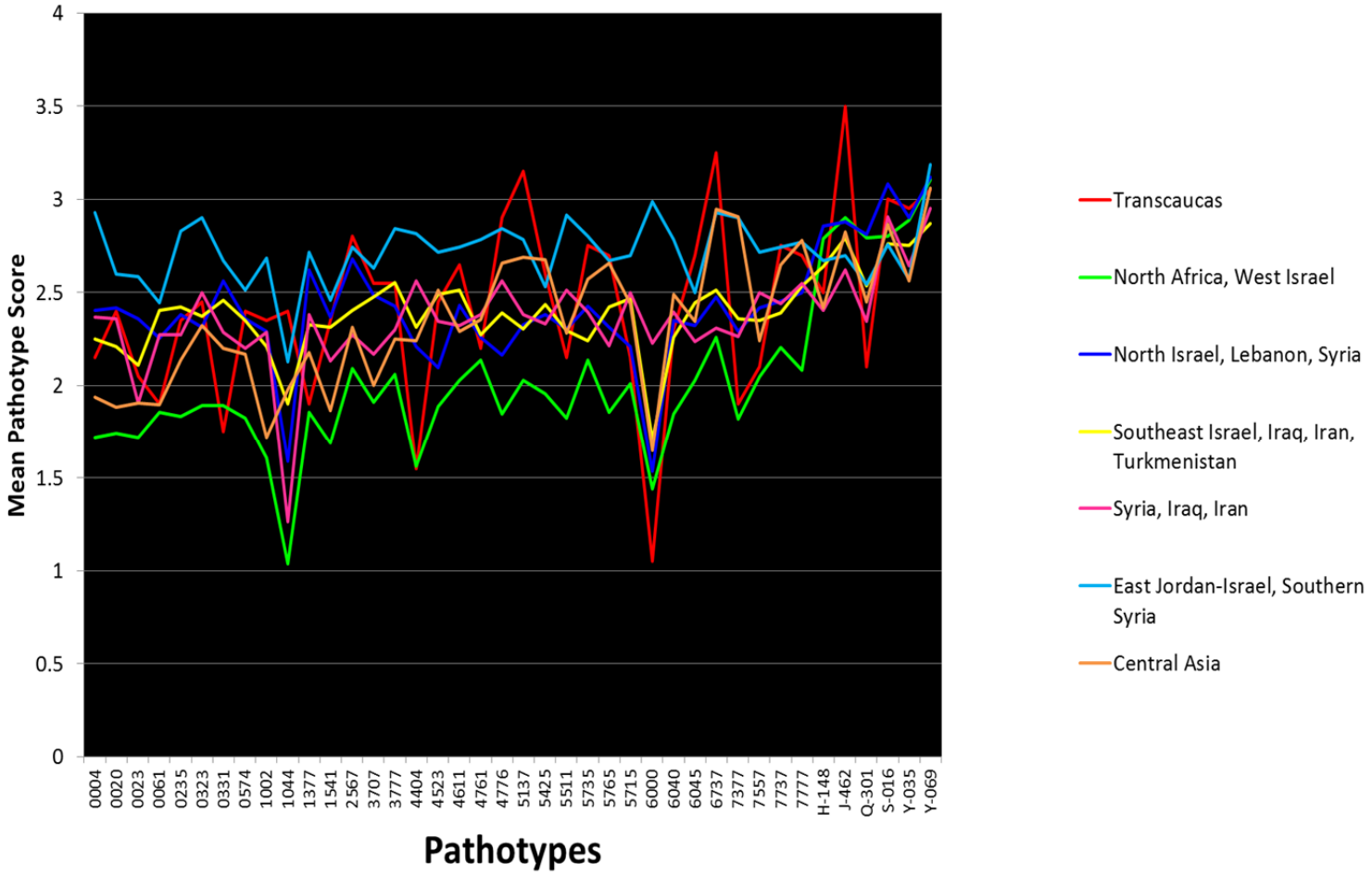


Figure 3. **Geographic locations of accessions, colored by subpopulation membership.** Sub-populations are roughly geographically grouped, as indicated by circles.

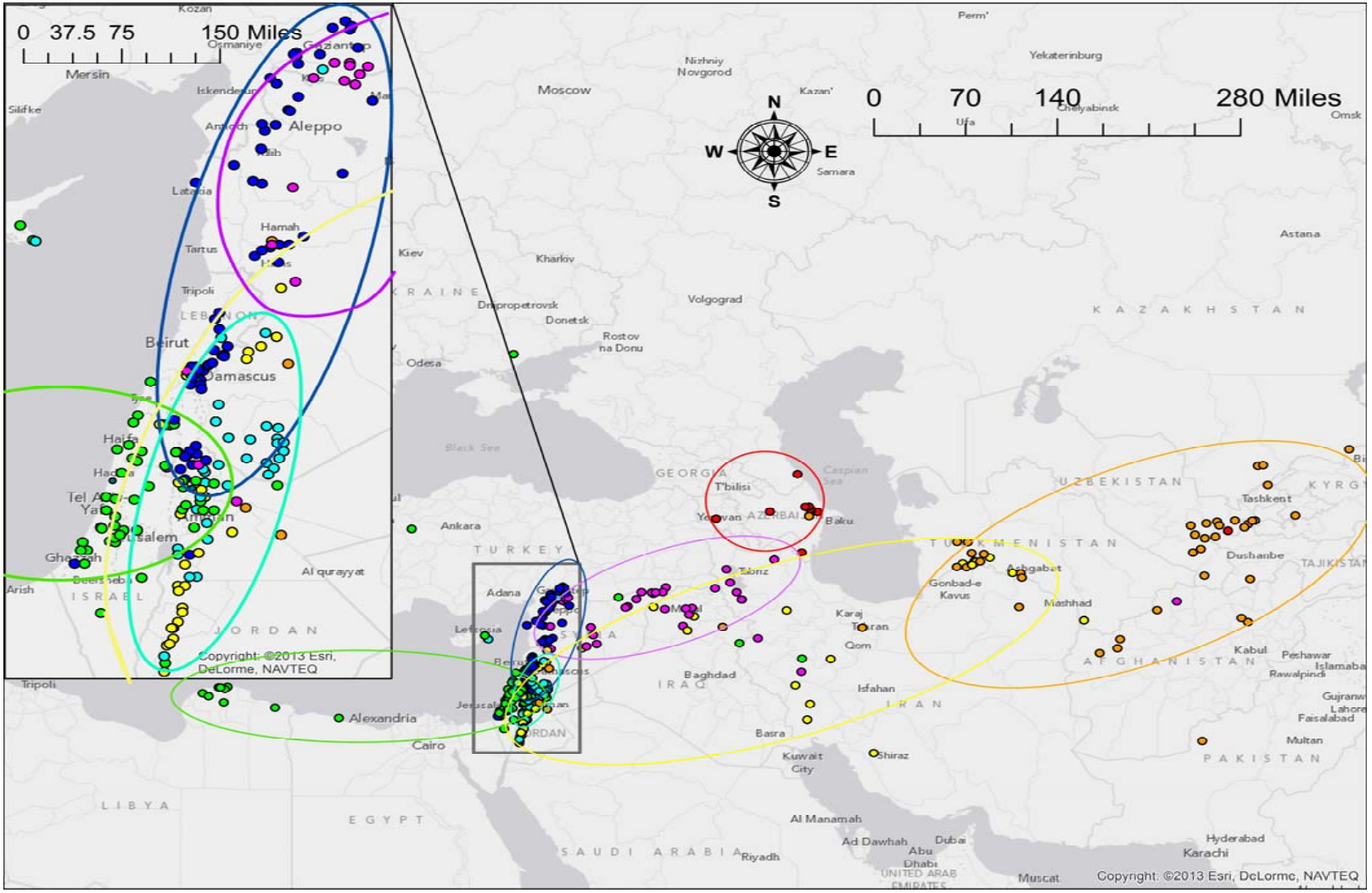


Figure 4. Barley genome map showing the positions of powdery mildew resistance loci identified in this study and in previous studies. Blue circles indicate loci found in this study, black triangles indicate relevant previously identified loci, bars indicate QTL intervals.

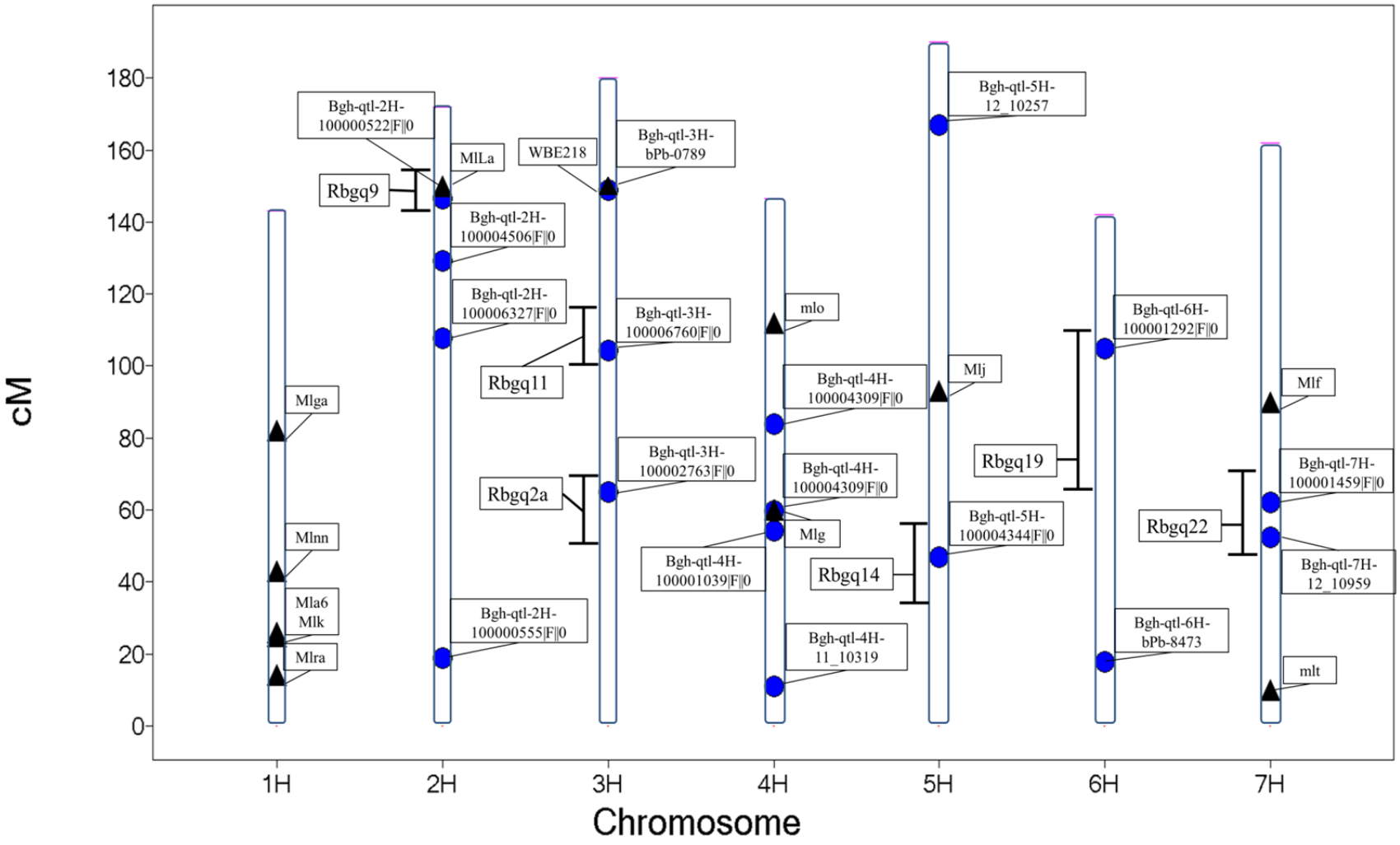


Figure 5. **Level of linkage disequilibrium found across all seven chromosomes in accessions of the Wild Barley Diversity Collection.**

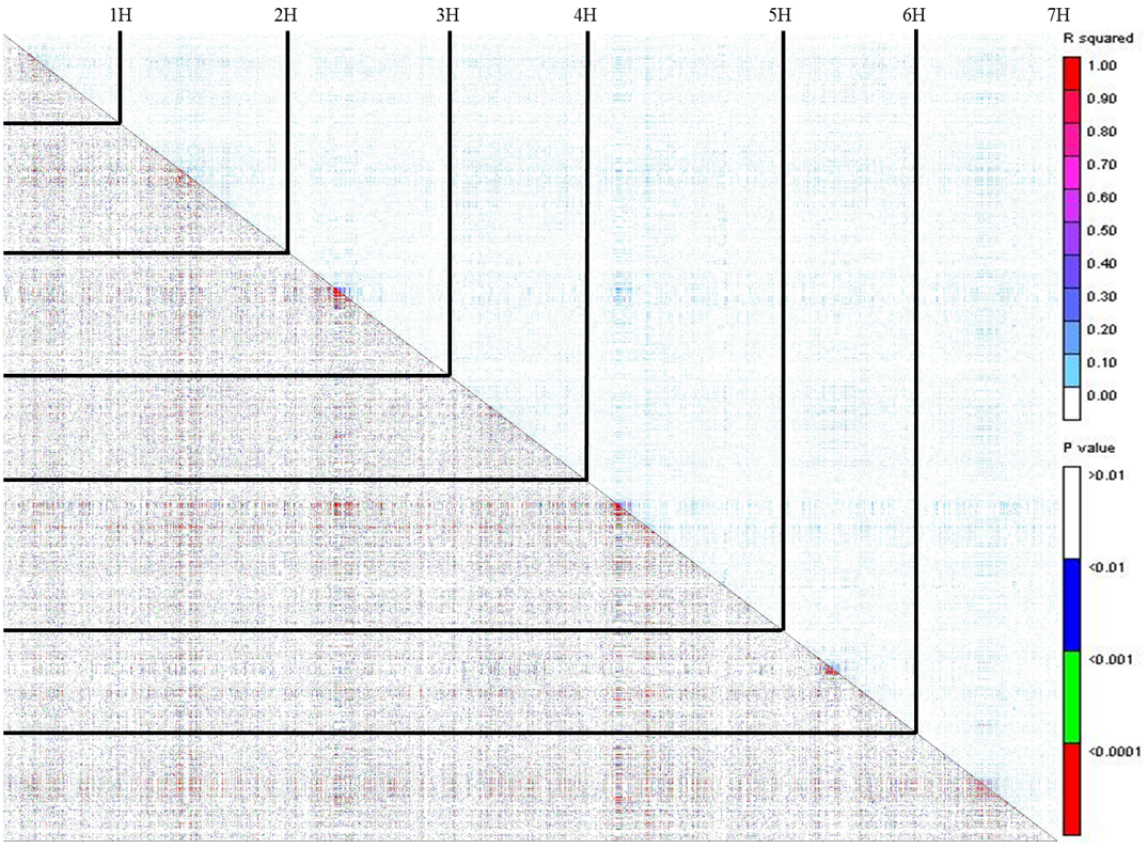
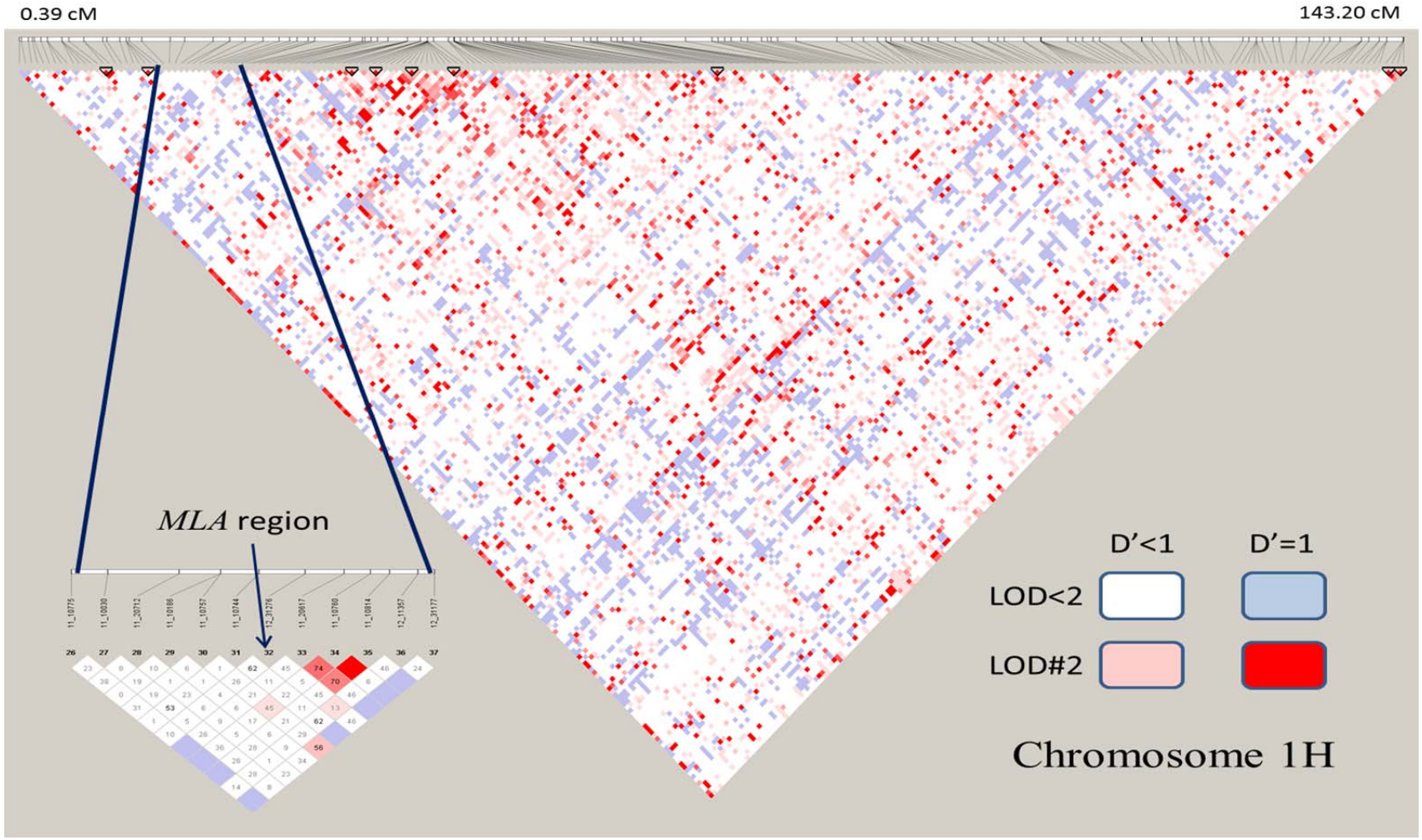


Figure 6**.** Level of l**inkage disequilibrium of chromosome 1H in accessions of the Wild Barley Diversity Collection showing an inset** of a 20-30 cM interval containing the *Mla* locus.



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Appendix

Table 2.1 : List of the Wild Barley Diversity Collection accessions used in this study and their accompanying passport and ecogeographic data.

Accession	Country	Region	Long[1]	Lat[2]	Alt[3]	Preyr[4]	Tmaxyr[5]	Tminyr[6]	Ariyr[7]
WBDC 001	Syria	Idlib	36.7	36.22	354	575.65	22.60	11.59	0.426
WBDC 002	Syria	Aleppo	37.44	35.71	286	242.59	25.14	10.98	0.152
WBDC 004	Syria	Idlib	36.54	35.97	236	584	23.92	12.60	0.433
WBDC 005	Jordan	Irbid	35.92	32.45	662	439	20.69	12.08	0.325
WBDC 006	Jordan	Mafraq	36.17	32.30	753	316.48	22.48	10.06	0.229
WBDC 007	Jordan	Irbid	35.92	32.33	1026	570	20.08	10.15	0.441
WBDC 008	Jordan	Irbid	35.62	32.67	-203	339.79	28.63	15.40	0.219
WBDC 009	Jordan	Amman	35.75	31.53	730	311.58	23.51	10.48	0.222
WBDC 010	Afghanistan	Jowzjan	65.73	36.67	360	209.39	24.11	10.02	0.141
WBDC 011	Iraq	Arbil	43.52	36.00	241	423.79	28.30	13.47	0.261
WBDC 012	Afghanistan	Faryab	64.82	36.28	596	284.03	22.85	8.14	0.203
WBDC 013	Iraq	As Sulaymaniyah	44.83	35.53	701	594	25.38	12.59	0.400
WBDC 014	Afghanistan	Baghlan	69.00	35.75	2205	442.42	15.61	3.17	0.443
WBDC 015	Afghanistan	Herat	62.18	34.35	926	239.27	23.21	8.33	0.156
WBDC 016	Iran	Khuzestan	48.85	32.00	39	284.66	32.18	16.63	0.141
WBDC 017	Syria	Dar'a	35.82	32.74	219	378	25.81	13.28	0.268
WBDC 018	Afghanistan	Badghis	63.12	34.98	940	289.46	22.07	7.27	0.210
WBDC 019	Iran	West Azerbaijan	45.72	36.75	1440	431.24	18.76	5.45	0.358
WBDC 020	Turkey	Urfa	40.03	36.85	373	372.7	25.06	11.32	0.245
WBDC 021	Iraq	Baqubah	45.60	34.80	424	531	27.46	12.86	0.338
WBDC 022	Turkey	Eskisehir	30.52	39.78	796				
WBDC 023	Iran	Lorestan	48.45	33.50	1769	464.88	19.48	6.26	0.350
WBDC 024	Iran	Khuzestan	48.72	31.28	24	185.33	29.26	15.20	0.088

WBDC 025	Pakistan	Baluchistan	66.90	30.30	1553	239.2	24.31	7.43	0.171
WBDC 026	Tajikistan	Kulyab	69.10	37.65	590	360.93	22.89	8.29	0.263
WBDC 027	Azerbaijan	Agdam Rayonu	47.00	40.50	89				
WBDC 028	Israel	Hamerkaz	34.93	31.95	113	573.41	25.92	13.96	0.424
WBDC 029	Israel	Hadarom	34.62	31.58	58	378	26.18	14.24	0.264
WBDC 030	Israel	Hazafon	35.18	33.08	99	697	23.98	14.86	0.539
WBDC 031	Israel	Hefa	35.08	32.75	16	531	25.85	15.68	0.395
WBDC 032	Israel	Hazafon	35.13	33.00	36	582.89	25.52	15.70	0.430
WBDC 033	Israel	Hadarom	35.22	31.27	517	311.72	24.48	12.12	0.221
WBDC 034	Israel	Jerusalem Tel Gezer	34.92	31.85	215				
WBDC 035	Israel	Yaar Hanassi	34.59	31.43	317				
WBDC 036	Afghanistan	Herat	63.00	34.57	2253	310.69	15.60	1.15	0.305
WBDC 037	Israel	Biriyya	35.50	32.98	874				
WBDC 038	Israel	West Bank	35.21	46.77	782				
WBDC 039	Jordan	Balqa	35.83	32.03	1001	553.98	21.79	10.40	0.420
WBDC 040	Israel	Hefa	34.95	32.70	36				
WBDC 041	Israel	Hadarom	34.57	31.67	63	412.12	25.83	14.18	0.292
WBDC 042	Israel	Hazafon	35.53	32.97	493	732.47	20.89	12.17	0.575
WBDC 043	Israel	Hazafon	35.58	32.98	237	566.13	24.80	14.03	0.410
WBDC 044	Israel	Tel Aviv	34.83	32.17	28	586.52	25.33	14.35	0.442
WBDC 045	Jordan	Amman	35.95	31.70	717	238.18	23.44	9.52	0.170
WBDC 046	Jordan	Irbid	35.90	32.48	709	416.33	20.51	12.56	0.308
WBDC 047	Jordan	Irbid	36.07	32.53	587	282.02	22.96	11.55	0.202
WBDC 048	Turkey	Hakkari	44.48	37.25	1396	631.09	17.54	6.02	0.562
WBDC 049	Turkey	Hakkari	44.48	37.25	1396	631.09	17.54	6.02	0.562
WBDC 050	Syria	Homs	38.87	35.16	473	169.02	25.10	10.78	0.096
WBDC 051	Syria	Homs	39.03	34.76	429	134.6	25.87	11.37	0.073
WBDC 052	Jordan	Mafraq	36.02	32.32	791	437.56	21.78	10.87	0.322

WBDC 053	Pakistan	Baluchistan	66.90	30.30	1553	239.2	24.31	7.43	0.171
WBDC 054	Syria	Homs	38.36	34.57	388	131.58	26.06	11.67	0.071
WBDC 055	Syria	Lattakia	35.82	35.61	61	836.51	23.45	14.92	0.715
WBDC 056	Turkey	Gaziantep	37.12	36.72	728	498.78	22.85	10.59	0.364
WBDC 057	Syria	Damascus	36.84	33.65	647	126.31	24.49	8.94	0.084
WBDC 058	Cyprus	Famagusta	34.02	34.99	67	449	24.44	13.69	0.367
WBDC 059	Cyprus	Famagusta	34.05	34.98	120	446.6	24.66	13.99	0.363
WBDC 060	Egypt	Marsa Matruh	27.17	31.35	26	128.7	23.67	15.45	0.079
WBDC 061	Syria	Idlib	36.58	36.16	725	660.26	21.33	10.79	0.516
WBDC 062	Syria	Aleppo	36.84	36.38	202	496.76	24.11	12.59	0.351
WBDC 063	Syria	Aleppo	36.64	36.72	312	706.38	23.36	12.76	0.542
WBDC 064	Syria	Idlib	36.24	35.80	761	1011	20.78	10.72	0.863
WBDC 065	Syria	Hama	36.66	34.98	394	386	23.93	11.44	0.279
WBDC 066	Syria	Damascus	36.40	33.78	1815	647.9	15.63	4.45	0.578
WBDC 067	Syria	Damascus	36.38	33.70	1331	486	18.61	5.57	0.397
WBDC 068	Syria	Sweida	36.72	32.82	1360	343.16	20.75	8.27	0.257
WBDC 069	Syria	Damascus	36.13	33.75	1307	592.19	19.41	6.30	0.485
WBDC 070	Syria	Lattakia	35.82	35.61	61	836.51	23.45	14.92	0.715
WBDC 072	Libya	Al Bayda	21.63	32.78	355	391.22	21.87	11.86	0.309
WBDC 073	Libya	Al Bayda	21.87	32.08	202	269.11	24.23	12.79	0.193
WBDC 074	Libya	Al Qubbah	22.05	32.80	686	564.1	21.40	10.49	0.462
WBDC 075	Libya	Shahhat	21.92	32.70	745	561.16	21.43	10.37	0.458
WBDC 078	Syria	Al Hasakah	40.86	36.77	364	364	25.47	11.77	0.235
WBDC 079	Jordan	Irbid	35.88	32.25	433	317.32	23.78	12.43	0.231
WBDC 080	Jordan	Irbid	35.78	32.53	613	479.01	20.99	13.21	0.358
WBDC 081	Jordan	Irbid	35.65	32.48	359	330	25.87	14.08	0.238
WBDC 082	Jordan	Irbid	35.78	32.27	923	640.53	21.39	11.73	0.485
WBDC 083	Jordan	Zarqa	35.92	32.17	688	352	23.47	11.67	0.258

WBDC 085	Jordan	Balqa	35.65	31.98	385	249.75	27.70	13.99	0.176
WBDC 089	Jordan	Amman	35.80	31.83	638	440.18	23.39	10.61	0.322
WBDC 092	Jordan	Zarqa	36.27	32.12	595	122.06	24.79	10.19	0.086
WBDC 093	Jordan	Amman	36.37	32.05	626	112.73	24.82	10.06	0.078
WBDC 094	Jordan	Karak	35.70	31.42	278	107	28.28	13.76	0.069
WBDC 095	Jordan	Karak	35.62	31.18	599	235.84	23.76	11.49	0.163
WBDC 097	Jordan	Karak	35.83	31.28	870	270.43	22.81	10.37	0.190
WBDC 100	Jordan	Tafila	35.62	30.77	1369	234	21.95	10.90	0.168
WBDC 101	Jordan	Tafila	35.57	30.70	1077	310.5	19.86	8.30	0.248
WBDC 102	Jordan	Ma'an	35.57	30.58	837	196.65	21.19	9.53	0.148
WBDC 103	Jordan	Ma'an	35.47	30.20	1693	144.7	18.96	7.21	0.123
WBDC 104	Jordan	Karak	35.62	31.18	599	235.84	23.76	11.49	0.163
WBDC 105	Jordan	Irbid	35.83	32.65	570	409.7	23.40	12.92	0.301
WBDC 106	Syria	Homs	36.74	34.94	401	354.4	23.92	11.15	0.253
WBDC 107	Syria	Homs	36.73	34.75	489	381.25	22.75	10.81	0.278
WBDC 108	Syria	Damascus	36.71	33.94	1364	386.13	19.19	5.99	0.291
WBDC 109	Syria	Sweida	36.72	32.77	1438	400.32	18.96	7.30	0.317
WBDC 110	Syria	Sweida	36.79	32.78	1489	374.35	19.40	7.48	0.290
WBDC 111	Syria	Damascus	36.54	33.84	1395	449.58	18.57	5.55	0.357
WBDC 112	Syria	Damascus	36.59	33.98	1646	513.25	17.46	5.19	0.419
WBDC 113	Turkmenistan	Ashkhabad	58.17	37.92	695	278	19.67	7.61	0.220
WBDC 115	Turkmenistan	Krasnovodsk	55.87	38.33	209	266	24.11	10.19	0.195
WBDC 116	Turkmenistan	Krasnovodsk	56.38	38.32	869	390.76	20.83	8.05	0.320
WBDC 117	Turkmenistan	Krasnovodsk	56.05	38.17	390	320.3	23.27	9.48	0.244
WBDC 119	Uzbekistan	Dzhizak	67.58	40.08	604	354	20.07	7.06	0.284
WBDC 120	Tajikistan	Dushanbe	67.50	39.47	1027	390.02	19.97	6.81	0.331
WBDC 121	Iran	Fars	51.78	29.72	1341				
WBDC 122	Iran	Khuzestan	48.17	32.87	888	378	27.48	13.81	0.225

WBDC 123	Iran	Khorasan	58.47	36.42	1396	292	19.58	8.67	0.227
WBDC 124	Iran	Kermanshahan	46.45	35.02	2270	565.08	15.81	2.56	0.508
WBDC 125	Uzbekistan	Kashkadar'ya	66.47	38.80	513	354	23.40	9.78	0.254
WBDC 126	Lebanon	Saida	35.32	33.45	190	728.38	24.48	15.47	0.548
WBDC 127	Syria	Sweida	36.73	32.94	1045	277.95	22.32	8.64	0.197
WBDC 128	Syria	Damascus	36.17	33.84	1738	569.02	19.49	6.16	0.467
WBDC 129	Syria	Sweida	36.42	32.81	750	279.81	23.77	9.81	0.192
WBDC 130	Syria	Dar'a	36.18	32.83	534	315	24.46	10.85	0.217
WBDC 131	Syria	Dar'a	36.04	33.01	691	449	22.77	10.68	0.318
WBDC 132	Lebanon	Rachaiya	35.87	33.47	1542	870.4	18.09	7.66	0.720
WBDC 133	Lebanon	Biqaa Al Gharbi	35.82	33.62	960	938	19.20	8.45	0.752
WBDC 134	Lebanon	Biqaa Al Gharbi	35.77	33.62	1062	944.56	20.12	9.40	0.734
WBDC 135	Lebanon	Biqaa Al Gharbi	35.72	33.52	894	1081.2	19.83	9.93	0.841
WBDC 136	Lebanon	Rachaiya	35.77	33.52	1009	1039	18.82	8.78	0.836
WBDC 137	Lebanon	Rachaiya	35.82	33.45	1162	878.83	19.47	8.98	0.687
WBDC 138	Lebanon	Hasbaiya	35.76	33.42	1274	936.15	19.95	9.91	0.718
WBDC 139	Lebanon	Baalbek	36.10	33.93	1025	546.76	22.20	7.84	0.415
WBDC 140	Lebanon	Baalbek	36.08	34.20	1622	1017.4	14.13	4.81	1.006
WBDC 141	Lebanon	Baalbek	36.03	34.13	1371	1020.3	14.65	5.09	0.992
WBDC 142	Lebanon	Baalbek	36.08	34.02	1029	615.76	22.29	8.21	0.471
WBDC 143	Lebanon	Rachaiya	35.90	33.57	1574	919.33	16.34	6.27	0.822
WBDC 145	Lebanon	Zahle	36.02	33.80	1040	551.76	21.87	7.78	0.418
WBDC 146	Iran	West Azerbaijan	45.70	36.75	1648	438.84	17.61	4.67	0.379
WBDC 147	Iran	West Azerbaijan	45.17	37.50	1301	342	17.65	4.71	0.306
WBDC 148	Iran	West Azerbaijan	45.47	37.07	1381	381.47	18.13	4.68	0.328
WBDC 149	Iran	West Azerbaijan	45.00	38.08	1916	330.13	14.69	2.31	0.342
WBDC 150	Iran	East Azerbaijan	47.20	38.50	1449	309.66	15.95	2.90	0.320
WBDC 151	Syria	Aleppo	36.95	36.52	534	518.57	22.23	10.75	0.376

WBDC 152	Iran	Tehran	51.25	35.50	1014	179.57	23.23	9.97	0.123
WBDC 153	Iran	Markazi	49.80	34.08	1660	310.11	20.24	5.67	0.225
WBDC 154	Iraq	Mosul	43.00	35.58	219	344.08	28.26	13.64	0.206
WBDC 155	Iraq	Mosul	42.17	36.33	379	472.67	25.93	13.67	0.305
WBDC 156	Iraq	Mosul	41.65	36.42	549	468	24.33	12.92	0.310
WBDC 157	Iraq	Mosul	43.42	36.38	475	533.44	27.23	12.49	0.344
WBDC 158	Iraq	Mosul	43.20	35.37	229	331.46	28.16	13.68	0.197
WBDC 159	Syria	Sweida	36.79	32.68	1539	387.17	18.65	7.13	0.308
WBDC 160	Syria	Sweida	36.60	32.96	842	255.11	23.48	9.14	0.176
WBDC 161	Syria	Aleppo	37.58	36.65	628	399.01	23.11	9.80	0.280
WBDC 164	Syria	Al Hasakah	41.64	37.01	402	517	25.55	13.09	0.352
WBDC 165	Syria	Al Hasakah	42.21	37.29	337	680.34	26.33	13.21	0.503
WBDC 166	Syria	Al Hasakah	40.46	36.45	608	328.97	24.91	11.15	0.211
WBDC 167	Syria	Al Hasakah	40.36	36.40	694	383.47	23.48	10.44	0.253
WBDC 168	Lebanon	Biqaa Al Gharbi	35.72	33.57	948	885.45	20.87	10.58	0.674
WBDC 169	Lebanon	Rachaiya	35.78	33.50	943	779.04	21.12	10.47	0.584
WBDC 170	Lebanon	Rachaiya	35.95	33.63	1522	816	17.80	6.73	0.695
WBDC 171	Lebanon	Rachaiya	35.85	33.58	1065	773	20.46	9.02	0.593
WBDC 173	Iran	Hamadan	48.48	34.10	1791	396.52	19.00	4.96	0.294
WBDC 174	Iran	Kordestan	47.77	36.27	1655	348.07	17.60	3.40	0.294
WBDC 175	Iran	Kordestan	47.77	36.27	1655	348.07	17.60	3.40	0.294
WBDC 177	Iraq	Mosul	43.13	36.35	260	462.44	27.86	12.33	0.291
WBDC 178	Iraq	Mosul	43.28	36.08	266	428.57	27.99	12.87	0.266
WBDC 179	Libya	Al Marj	20.90	32.50	294	322.47	22.97	13.08	0.255
WBDC 180	Libya	Tubruq	24.23	31.83	155	131.45	24.38	13.88	0.086
WBDC 181	Jordan	Zarqa	36.02	32.02	629	242.01	24.01	10.8	0.175
WBDC 182	Jordan	Mafraq	36.72	32.30	1014	157.55	22.71	9.47	0.110
WBDC 183	Jordan	Irbid	35.72	32.63	354	323	27.13	14.22	0.223

WBDC 184	Libya	Al Marj	21.17	32.37	482	347.62	22.90	11.95	0.275
WBDC 185	Libya	Al Bayda	21.72	32.77	599	501.74	21.40	10.91	0.404
WBDC 186	Turkey	Gaziantep	37.35	36.88	692	486	22.72	10.00	0.363
WBDC 187	Turkey	Gaziantep	37.46	36.69	554	411.98	23.37	10.23	0.290
WBDC 188	Turkey	Gaziantep	37.52	36.88	625	455.23	23.11	10.02	0.334
WBDC 189	Turkey	Gaziantep	37.63	36.76	526	402.97	23.76	10.27	0.283
WBDC 190	Turkey	Gaziantep	37.52	36.85	606	440.89	23.33	10.18	0.320
WBDC 191	Turkey	Gaziantep	37.72	36.84	514	407.78	23.91	10.28	0.289
WBDC 192	Turkey	Gaziantep	37.61	37.04	719	486	22.58	9.60	0.370
WBDC 193	Turkey	Gaziantep	37.47	37.32	632	559.37	22.84	10.07	0.436
WBDC 194	Turkey	Gaziantep	37.19	36.97	996	601	20.27	8.25	0.492
WBDC 195	Turkey	Gaziantep	37.22	36.81	705	509.65	22.48	10.05	0.381
WBDC 196	Turkey	Gaziantep	36.93	36.99	1136	683.2	18.72	7.23	0.590
WBDC 197	Syria	Aleppo	37.77	36.48	536	330	23.85	9.97	0.221
WBDC 198	Syria	Homs	36.47	34.82	506	542.54	22.02	11.40	0.413
WBDC 199	Syria	Homs	36.64	34.91	386	388.64	23.70	11.61	0.281
WBDC 200	Syria	Hama	36.66	34.94	400	386.7	23.68	11.35	0.280
WBDC 201	Syria	Idlib	36.89	35.56	459	386	23.78	10.59	0.270
WBDC 202	Syria	Idlib	36.55	35.60	637	618	22.62	10.64	0.476
WBDC 203	Syria	Idlib	36.45	35.63	732	815.27	20.71	10.09	0.669
WBDC 204	Turkmenistan	Ashkhabad	57.12	38.58	111	199.62	22.52	10.42	0.143
WBDC 205	Russia	Dagestan	48.27	42.05	73				
WBDC 206	Syria	Sweida	36.60	32.55	1045	315	21.83	9.26	0.230
WBDC 207	Uzbekistan	Fergana	71.18	40.34	557	132.02	20.28	8.10	0.105
WBDC 208	Uzbekistan	Tashkent	69.91	41.61	817	701.27	19.24	6.34	0.625
WBDC 209	Uzbekistan	Dzhizak	68.40	40.13	386	338.62	20.96	7.90	0.265
WBDC 210	Uzbekistan	Dzhizak	67.68	39.93	835	376.76	19.06	6.64	0.317
WBDC 211	Uzbekistan	Dzhizak	68.08	39.71	1539	384.73	16.74	4.42	0.378

WBDC 212	Uzbekistan	Dzhizak	67.09	40.01	695	340	20.21	6.82	0.273
WBDC 213	Uzbekistan	Samarkand	66.56	39.55	718	391.08	21.13	7.80	0.306
WBDC 214	Uzbekistan	Samarkand	67.02	39.40	987	422.89	21.21	7.36	0.347
WBDC 215	Turkmenistan	Krasnovodsk	56.29	38.28	885	406.92	19.97	7.47	0.342
WBDC 216	Turkmenistan	Ashkhabad	56.85	38.73	62	227.21	23.04	10.46	0.160
WBDC 217	Armenia	Yerevan	44.53	40.20	1278	358.39	15.63	4.48	0.355
WBDC 218	Kazakhstan	Dzambull	73.66	43.06	602	376	16.35	3.19	0.386
WBDC 219	Kazakhstan	Chimkent	69.46	42.39	414	443.35	19.42	6.31	0.380
WBDC 220	Kazakhstan	Chimkent	69.70	42.42	522	525.08	18.82	5.86	0.465
WBDC 221	Tajikistan	Dushanbe	69.33	40.13	433	340	19.61	7.76	0.269
WBDC 222	Tajikistan	Dushanbe	69.33	40.13	433	340	19.61	7.76	0.269
WBDC 223	Tajikistan	Dushanbe	69.00	39.97	864				
WBDC 224	Tajikistan	Dushanbe	68.84	39.85	1212				
WBDC 225	Tajikistan	Dushanbe	69.20	40.12	487	365.34	19.58	7.52	0.295
WBDC 227	Azerbaijan	Lankaran	48.47	38.80	755	632.23	16.31	6.57	0.661
WBDC 228	Azerbaijan	Abseron Peninsula	49.19	40.49	550	231.11	16.07	7.60	0.257
WBDC 229	Azerbaijan	Qobustan	48.89	40.53	799	357.91	14.97	6.24	0.426
WBDC 230	Azerbaijan	Qobustan	48.88	40.61	1012	394.03	13.91	5.23	0.493
WBDC 231	Azerbaijan	Samaxi	48.80	40.71	1196	461.36	13.08	4.37	0.601
WBDC 232	Azerbaijan	Samaxi	48.63	40.70	1181	535.76	13.52	4.67	0.682
WBDC 233	Afghanistan	Baghlan	68.70	35.93	813	285	23.10	9.30	0.229
WBDC 234	Cyprus	Famagusta	33.88	35.15	13	390	25.10	13.7	0.315
WBDC 235	Jordan	Amman	35.90	32.02	896	508	21.20	9.70	0.389
WBDC 236	Jordan	Amman	35.62	31.60	218	125	28.90	14.2	0.082
WBDC 237	Jordan	Amman	35.62	31.53	791	315	24.40	11.4	0.222
WBDC 238	Jordan	Amman	35.85	31.55	701	164	24.60	10.8	0.113
WBDC 240	Jordan	Amman	35.94	31.88	874	379	22.70	9.90	0.279
WBDC 241	Jordan	Amman	36.22	31.78	761	133	23.70	9.50	0.094

WBDC 242	Jordan	Balqa	35.87	32.12	814	343	23.90	11.90	0.251
WBDC 243	Jordan	Balqa	35.67	32.03	312	169	28.90	14.70	0.117
WBDC 244	Jordan	Balqa	35.71	32.08	1051	645	21.10	10.50	0.500
WBDC 245	Jordan	Irbid	35.87	32.17	698	466	22.80	11.60	0.345
WBDC 246	Jordan	Irbid	35.75	32.68	430	380	25.80	13.70	0.271
WBDC 247	Jordan	Irbid	35.70	32.37	398	384	24.60	13.70	0.281
WBDC 248	Jordan	Irbid	35.85	32.53	687	480	19.70	12.70	0.359
WBDC 250	Jordan	Irbid	35.93	32.58	564	385	21.40	12.50	0.283
WBDC 252	Jordan	Irbid	35.68	32.28	367	272	26.90	14.20	0.194
WBDC 253	Jordan	Irbid	35.82	32.35	1053	620	19.30	9.80	0.495
WBDC 254	Jordan	Irbid	35.70	32.62	206	421	24.30	13.60	0.308
WBDC 255	Jordan	Irbid	35.67	32.35	606	508	23.10	13.30	0.379
WBDC 256	Jordan	Irbid	35.63	32.33	159	266	28.00	14.70	0.187
WBDC 257	Jordan	Karak	35.70	31.18	746	382	23.10	9.90	0.277
WBDC 258	Jordan	Karak	35.75	31.28	910	343	22.40	10.20	0.245
WBDC 259	Jordan	Karak	35.63	31.12	1158	363	22.50	10.30	0.263
WBDC 260	Jordan	Ma'an	35.50	30.50	959	114	23.60	11.30	0.079
WBDC 261	Jordan	Ma'an	35.53	30.52	1496	272	18.90	7.20	0.228
WBDC 262	Jordan	Ma'an	35.48	30.30	1521	150	19.90	8.00	0.122
WBDC 263	Jordan	Ma'an	35.47	30.38	1105	150	22.10	9.90	0.111
WBDC 265	Jordan	Tafila	35.67	30.93	747	330	21.70	10.40	0.241
WBDC 266	Jordan	Tafila	35.67	30.88	1178	330	21.20	9.80	0.247
WBDC 267	Jordan	Tafila	35.55	30.70	1113	134	23.30	11.50	0.093
WBDC 268	Jordan	Zarqa	36.76	31.75	527	71	26.60	11.40	0.044
WBDC 269	Lebanon		35.87	33.42	2692	1072	9.30	1.20	1.251
WBDC 270	Israel	Hadarom	34.87	31.68	215	445	25.90	13.60	0.321
WBDC 271	Israel	Hadarom	34.48	31.43	54	312	26.40	14.50	0.213
WBDC 274	Israel	Hadarom	34.77	30.87	481	168	24.50	12.60	0.116

WBDC 275	Israel	Hadarom	34.55	31.58	12	379	26.10	14.40	0.264
WBDC 276	Israel	Hamerkaz	34.90	32.35	54	590	25.50	14.90	0.446
WBDC 277	Israel	Hamerkaz	34.93	32.15	27	584	25.70	14.40	0.438
WBDC 278	Israel	Hamerkaz	34.77	31.83	46	491	25.90	14.10	0.355
WBDC 279	Israel	Hamerkaz	34.78	31.82	73	490	25.80	14.00	0.355
WBDC 280	Israel	Hamerkaz	34.83	32.02	29	579	25.40	14.00	0.430
WBDC 281	Israel	Hazafon	35.58	33.03	128	528	25.50	14.40	0.374
WBDC 282	Israel	Hazafon	35.22	32.68	110	543	25.40	15.00	0.403
WBDC 283	Israel	Hazafon	35.12	32.60	261	601	24.60	14.70	0.463
WBDC 284	Israel	Hazafon	35.60	32.67	-185	352	28.30	15.30	0.228
WBDC 285	Israel	Hazafon	35.45	32.98	725	721	22.40	13.30	0.561
WBDC 286	Israel	Hazafon	35.47	32.97	417	651	23.30	13.70	0.495
WBDC 287	Israel	Hazafon	35.50	32.50	-116	367	27.90	15.00	0.252
WBDC 288	Israel	Hazafon	35.42	32.98	1003	825	19.90	11.50	0.675
WBDC 289	Israel	Hazafon	35.48	32.52	-92	382	27.70	14.90	0.261
WBDC 290	Israel	Jerusalem	34.93	31.67	369	474	25.20	13.10	0.348
WBDC 291	Israel	Jerusalem	34.92	31.72	195	456	26.20	13.80	0.328
WBDC 292	Israel	Jerusalem	35.02	31.80	328	513	25.50	13.40	0.380
WBDC 293	Israel	Jerusalem	35.17	31.80	562	537	24.60	12.70	0.402
WBDC 294	Israel	Jerusalem	34.98	31.75	218	469	26.30	13.80	0.338
WBDC 295	Syria	Al Hasakah	42.07	37.08	491	669	24.50	12.80	0.485
WBDC 296	Syria	Al Hasakah	41.56	37.06	445	508	25.40	12.90	0.346
WBDC 297	Syria	Al Hasakah	41.75	37.06	559	594	24.70	12.70	0.420
WBDC 298	Syria	Al Hasakah	41.09	37.08	485	464	24.60	11.80	0.314
WBDC 299	Syria	Aleppo	36.71	36.66	495	677	22.40	11.60	0.519
WBDC 300	Syria	Aleppo	36.86	36.37	254	507	23.30	11.80	0.361
WBDC 302	Syria	Damascus	35.88	33.37	1397	917	15.80	6.30	0.829
WBDC 303	Syria	Damascus	36.11	33.74	1252	548	20.50	6.90	0.432

WBDC 304	Syria	Damascus	35.96	33.64	1494	839	16.80	6.20	0.741
WBDC 305	Syria	Damascus	36.00	33.66	1642	753	18.00	6.50	0.639
WBDC 306	Syria	Damascus	36.13	33.73	1479	667	17.70	5.60	0.577
WBDC 307	Syria	Damascus	36.07	33.20	800	474	22.30	9.80	0.337
WBDC 308	Syria	Dar'a	36.38	32.60	737	263	23.40	9.90	0.183
WBDC 309	Syria	Hama	37.01	35.03	547	299	24.40	10.30	0.205
WBDC 310	Syria	Homs	36.92	34.54	806	315	21.70	9.00	0.223
WBDC 311	Syria	Homs	38.56	34.65	467	135	25.40	11.10	0.073
WBDC 312	Syria	Homs	36.76	34.47	719	337	21.90	9.60	0.243
WBDC 314	Syria	Homs	36.55	34.88	360	439	23.30	11.90	0.323
WBDC 315	Syria	Homs	36.85	34.94	454	316	24.20	10.90	0.222
WBDC 316	Syria	Idlib	36.55	36.23	403	650	22.70	12.10	0.496
WBDC 317	Syria	Raqqa	38.76	35.52	314	169	25.70	11.10	0.098
WBDC 318	Syria	Sweida	36.74	32.60	1561	386	18.90	7.50	0.308
WBDC 319	Syria	Sweida	36.68	32.49	1221	316	21.00	8.80	0.237
WBDC 320	Syria	Sweida	36.62	32.42	1102	290	21.70	9.10	0.212
WBDC 323	Turkmenistan	Ahalskii	58.55	37.90	227	236	22.80	9.90	0.165
WBDC 324	Turkmenistan	Ahalskii	61.46	35.83	746	313	22.30	7.80	0.214
WBDC 326	Turkmenistan	Ashkhabad	58.60	37.72	660	279	20.40	8.10	0.215
WBDC 329	Turkmenistan	Balkan	55.60	38.18	136	248	24.50	10.60	0.180
WBDC 330	Turkmenistan	Balkansky	56.29	38.77	407	289	21.50	8.90	0.220
WBDC 331	Turkmenistan	Balkansky	56.11	39.24	51	184	22.40	10.00	0.126
WBDC 332	Turkmenistan	Garygalla	56.42	38.43	439	330	22.50	9.20	0.255
WBDC 333	Turkmenistan	Garygalla	56.49	38.41	526	358	21.90	8.80	0.282
WBDC 334	Turkmenistan	Garygalla	56.31	38.27	1055	437	18.90	6.80	0.379
WBDC 335	Turkmenistan	Kazanjik	55.62	39.25	63	181	22.30	10.10	0.126
WBDC 336	Turkmenistan	Krasnovodsk	56.68	38.42	691	379	20.70	8.30	0.308
WBDC 337	Turkey	Gaziantep	37.52	37.23	796	548	22.10	9.40	0.434

WBDC 338	Turkey	Gaziantep	37.54	37.27	568	529	23.40	10.50	0.403
WBDC 340	Turkey	Gaziantep	37.33	37.25	852	590	21.10	8.70	0.484
WBDC 341	Turkey	Gaziantep	36.93	36.95	968	651	21.50	9.90	0.516
WBDC 342	Turkey	Gaziantep	36.90	36.97	934	677	20.40	8.80	0.556
WBDC 343	Turkey	Gaziantep	36.95	36.87	564	617	23.00	11.20	0.465
WBDC 345	Uzbekistan	Kashkadar'ya	66.83	38.95	682	386	22.80	9.10	0.290
WBDC 346	Uzbekistan	Samarkand	66.37	39.92	484	313	21.30	8.00	0.236
WBDC 347	Uzbekistan	Surkhandar'ya	67.00	37.80	602	278	23.30	10.20	0.197
WBDC 348	Israel		35.00	32.44	425				
WBDC 349	Israel		35.14	32.14	400				
WBDC 350	Israel		35.30	32.29	-151				
WBDC 354	Israel	Tel Megido Plateau of Menashe	35.17	32.57	100	550			
WBDC 355	Transcaucasus region		48.81	40.31					

[1]Longitude
[2]Latitude
[3]Altitude
[4]Precipitation per year
[5]Yearly maximum temperature
[6]Yearly minimum temperature
[7]Average yearly aridity

Table 2.2 Summary of reactions of Wild Barley Diversity Collection accessions in response to 40 isolates of *Blumeria graminis* f. sp. *hordei*.

Accession Number[1]	# of pathotypes resisted[2]	Octal Code[3]	Postulated genes[4]
WBDC001	3 (7.5%)	77762777777771	
WBDC002	4 (10%)	77723677777771	
WBDC004	19 (47.5%)	50504503732671	
WBDC005	0 (0%)	77777777777771	
WBDC006	0 (0%)	77777777777771	
WBDC007	1 (2.5%)	77767777777771	
WBDC008	25 (62.5%)	23100241105661	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC009	13 (32.5%)	21335325177771	*Mlk1 Ml(Ru2)*
WBDC010	29 (72.5%)	36400013404100	*Mla3 Mla6*
WBDC011	1 (2.5%)	77767777777771	
WBDC012	0 (0%)	77777777777771	
WBDC013	0 (0%)	77777777777771	
WBDC014	20 (50%)	25325215045561	*Mla1 Mla3 Mla6 Mla12 Mlk1 MILa Mlg Mlat Ml(Ru2)*
WBDC015	30 (75%)	00000011045561	*Mla1 Mla3 Mla6 Mla12 Mlk1 MILa Mlg Mlat Ml(Ru2)*
WBDC016	17 (42.5%)	00000777777561	*Mla6*
WBDC017	0 (0%)	77777777777771	
WBDC018	1 (2.5%)	77767777777771	
WBDC019	22 (55%)	04120654064771	*Mlk1*
WBDC020	0 (0%)	77777777777771	
WBDC021	37 (92.5%)	00000000000441	*Mla1 Mla3 Mla6 Mla12 Mlk1 MIlLa Mlat Ml(Ru2)*
WBDC022	27 (67.5%)	15101030204461	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC023	0 (0%)	77777777777771	
WBDC024	24 (60%)	10404603720651	
WBDC025	15 (37.5%)	16402777651571	
WBDC026	34 (85%)	00000000041431	*Mla1 Mla3 Mla6 Mla12 Mlk1 MILa Mlg Mlat Ml(Ru2)*
WBDC027	4 (10%)	37767777775751	*Mla12 Mlk1 Ml(Ru2)*
WBDC028	0 (0%)	77777777777771	
WBDC029	5 (12.5%)	47377677677771	

WBDC030	9 (22.5%)	47767135277771	*Mlk1 Mlat*
WBDC031	30 (75%)	40020002004671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC032	19 (47.5%)	00075270165771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC033	4 (10%)	57367677777771	
WBDC034	0 (0%)	77777777777771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC035	25 (62.5%)	00000230076771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC036	13 (32.5%)	71531273165771	*Mlk1*
WBDC037	34 (85%)	00000000000671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC038	34 (85%)	00000000000671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC039	0 (0%)	77777777777771	
WBDC040	30 (75%)	20005500004251	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC041	1 (2.5%)	77777777377771	*Mlk1 Ml(Ru2)*
WBDC042	36 (90%)	00000000000251	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlg Mlat Ml(Ru2)*
WBDC043	34 (85%)	00000000000671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlg Mlat Ml(Ru2)*
WBDC044	4 (10%)	76727777775771	
WBDC045	8 (20%)	77325277177771	*Ml(Ru2)*
WBDC046	0 (0%)	77777777777771	
WBDC047	2 (5%)	77737777777761	
WBDC048	1 (2.5%)	77757777777771	*Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC049	18 (45%)	50504703732671	
WBDC050	2 (5%)	77767777777761	
WBDC051	7 (17.5%)	16777367177771	
WBDC052	1 (2.5%)	77767777777771	
WBDC053	38 (95%)	14000000000000	
WBDC054	2 (5%)	67777777677771	
WBDC055	4 (10%)	57367677777771	
WBDC056	0 (0%)	77777777777771	
WBDC057	3 (7.5%)	37767777377771	*Mlk1 MlLa Mlat Ml(Ru2)*
WBDC058	18 (45%)	00000757277771	*Mla6*
WBDC059	0 (0%)	77777777777771	
WBDC060	5 (12.5%)	77777657373761	
WBDC061	17 (42.5%)	00135076267771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC062	20 (50%)	50104603732671	
WBDC063	25 (62.5%)	03352000022671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC064	12 (30%)	15326653373771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC065	0 (0%)	77777777777771	
WBDC066	0 (0%)	77777777777771	*Mlat*

WBDC067	0 (0%)	77777777777771	
WBDC068	0 (0%)	77777777777771	
WBDC069	1 (2.5%)	77777777377771	
WBDC070	0 (0%)	77777777777771	
WBDC072	0 (0%)	77777777777771	*Mla1 Mla3 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC073	1 (2.5%)	37777777777771	
WBDC074	4 (10%)	77167777777731	*Mla1 Mla3 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC075	5 (12.5%)	36167777777771	
WBDC078	6 (15%)	36632777777771	
WBDC079	13 (32.5%)	46107657367751	*Mla1 Mla6 Mlk1 Ml(Ru2)*
WBDC080	17 (42.5%)	71143215067671	*Mlk1*
WBDC081	26 (65%)	13152010000671	*Mlk1 MlLa Mlg*
WBDC082	23 (57.5%)	25000623724660	*Mla1 Mla6 Mla9 Mla12 Mlk1 MlLa Mlg Mlat Ml(Ru2)*
WBDC083	23 (57.5%)	00000703732671	*Mla6*
WBDC085	38 (95%)	00000000000440	
WBDC089	38 (95%)	00000000004400	
WBDC092	1 (2.5%)	77767777777771	
WBDC093	4 (10%)	27767777773771	*MlLa*
WBDC094	6 (15%)	27767773376771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC095	22 (55%)	00063442145771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC097	1 (2.5%)	77777777767771	*Mla6*
WBDC100	0 (0%)	77777777777771	
WBDC101	0 (0%)	77777777777771	
WBDC102	0 (0%)	77777777777771	
WBDC103	0 (0%)	77777777777771	
WBDC104	12 (30%)	07166657367751	*Mlk1*
WBDC105	2 (5%)	77767777776771	
WBDC106	13 (32.5%)	24367677366321	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC107	2 (5%)	37767777777771	
WBDC108	0 (0%)	77777777777771	
WBDC109	0 (0%)	77777777777771	
WBDC110	1 (2.5%)	77777777777761	
WBDC111	0 (0%)	77777777777771	
WBDC112	0 (0%)	77777777777771	
WBDC113	16 (40%)	06337025265771	
WBDC115	10 (25%)	27776575261771	
WBDC116	4 (10%)	37767777375771	*Mlk1*

WBDC117	13 (32.5%)	67325275145771	
WBDC119	9 (22.5%)	26333775575771	
WBDC120	7 (17.5%)	77777265365771	*Mlk1 Mlg*
WBDC121	12 (30%)	77547664174571	
WBDC122	4 (10%)	77377765377771	
WBDC123	18 (45%)	00000357377771	*Mla1 Mla3 Mla6 Mlk1 MlLa Mlg Mlat Ml(Ru2)*
WBDC124	0 (0%)	77777777777771	
WBDC125	0 (0%)	77777777777771	*Mlg*
WBDC126	16 (40%)	61113665147671	
WBDC127	17 (42.5%)	71035615154671	
WBDC128	3 (7.5%)	75777775577771	
WBDC129	3 (7.5%)	75777677757771	
WBDC130	0 (0%)	77777777777771	
WBDC131	0 (0%)	77777777777771	
WBDC132	3 (7.5%)	37777775377771	
WBDC133	0 (0%)	77777777777771	
WBDC134	19 (47.5%)	70526725060670	
WBDC135	17 (42.5%)	46225275145761	
WBDC136	3 (7.5%)	77777774777770	
WBDC137	4 (10%)	76727777775771	
WBDC138	13 (32.5%)	32737566145671	
WBDC139	0 (0%)	77777777777771	
WBDC140	3 (7.5%)	77773777377761	*Mlk1*
WBDC141	0 (0%)	77777777777771	
WBDC142	9 (22.5%)	76777364176770	
WBDC143	1 (2.5%)	77777777577771	
WBDC145	10 (25%)	55173537536771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC146	1 (2.5%)	77777777777571	
WBDC147	10 (25%)	77747272176571	*Ml(Ru2)*
WBDC148	1 (2.5%)	77777377777771	
WBDC149	9 (22.5%)	66767172277771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC150	4 (10%)	34737777777771	
WBDC151	3 (7.5%)	77747377777771	
WBDC152	0 (0%)	77777777777771	
WBDC153	22 (55%)	47200274145701	
WBDC154	0 (0%)	77777777777771	
WBDC155	0 (0%)	77777777777771	

WBDC156	1 (2.5%)	77767777777771	
WBDC157	3 (7.5%)	77767777773571	
WBDC158	3 (7.5%)	77707777777771	
WBDC159	0 (0%)	77777777777771	
WBDC160	0 (0%)	77777777777771	
WBDC161	6 (15%)	36367377757771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC164	2 (5%)	77677777777761	
WBDC165	15 (37.5%)	17625375446661	*Mla7 Mla12 Mlk1 Mlg Mlat*
WBDC166	1 (2.5%)	77777777777761	
WBDC167	18 (45%)	42006337613571	
WBDC168	0 (0%)	77777777777771	
WBDC169	1 (2.5%)	77777677777771	
WBDC170	5 (12.5%)	77777267377571	*Mla6*
WBDC171	2 (5%)	77777757777671	
WBDC173	3 (7.5%)	76767777775771	
WBDC174	1 (2.5%)	77767777777771	
WBDC175	0 (0%)	77777777777771	
WBDC177	15 (37.5%)	00000777777771	*Mla6*
WBDC178	0 (0%)	77777777777771	
WBDC179	0 (0%)	77777777777771	
WBDC180	1 (2.5%)	77767777777771	
WBDC181	3 (7.5%)	76727777777771	
WBDC182	10 (25%)	60602777777771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC183	26 (65%)	00000260147671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC184	0 (0%)	77777777777771	
WBDC185	8 (20%)	24427777777771	*Mla6 Mla12*
WBDC186	33 (82.5%)	00021000000351	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC187	5 (12.5%)	37627775777771	*Mlk1 MlLa Mlat Ml(Ru2)*
WBDC188	31 (77.5%)	00021001004471	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC189	2 (5%)	77767777775771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC190	6 (15%)	37327777676771	
WBDC191	33 (82.5%)	04000000000671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC192	20 (50%)	03140353677500	*Mla6 Mla7 Mla12 MlLa Mlg*
WBDC193	7 (17.5%)	73473767277771	*Mla12 Mlk1 Ml(Ru2)*
WBDC194	1 (2.5%)	77767777777771	
WBDC195	0 (0%)	77777777777771	
WBDC196	3 (7.5%)	77767777367771	

WBDC197	5 (12.5%)	57277677377771	*Mlk1 Ml(Ru2)*
WBDC198	1 (2.5%)	37777777777771	
WBDC199	21 (52.5%)	70423512204671	*Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC200	8 (20%)	27747377677561	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC201	7 (17.5%)	27747377377571	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC202	3 (7.5%)	47767777777771	*Mla12*
WBDC203	20 (50%)	52421416114771	*Mla12*
WBDC204	1 (2.5%)	77777777377771	
WBDC205	17 (42.5%)	42111667146771	*Mla6 Mlk1 Ml(Ru2)*
WBDC206	1 (2.5%)	77777777377771	
WBDC207	2 (5%)	57777777377771	
WBDC208	20 (50%)	50114603332671	
WBDC209	0 (0%)	77777777777771	
WBDC210	1 (2.5%)	77777777377771	
WBDC211	11 (27.5%)	76457477167661	
WBDC212	16 (40%)	00000777777761	*Mla6*
WBDC213	11 (27.5%)	06333677374771	
WBDC214	4 (10%)	26777777377771	
WBDC215	9 (22.5%)	13667776175771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC216	21 (52.5%)	00000277165771	*Mla6*
WBDC217	6 (15%)	76607777377771	
WBDC218	0 (0%)	77777777777771	
WBDC219	0 (0%)	77777777777771	
WBDC220	0 (0%)	77777777777771	*Ml(Ru2)*
WBDC221	10 (25%)	12077775367771	
WBDC222	0 (0%)	77777777777771	
WBDC223	2 (5%)	77777777377761	
WBDC224	1 (2.5%)	37777777777771	
WBDC225	1 (2.5%)	77777777775771	
WBDC227	10 (25%)	77641677367561	
WBDC228	3 (7.5%)	77777676377771	
WBDC229	3 (7.5%)	27777775777771	
WBDC230	6 (15%)	75557675776771	
WBDC231	2 (5%)	77777577377771	
WBDC232	7 (17.5%)	74677667676771	
WBDC233	18 (45%)	05325235144771	*Mla1 Mla6 Mla12 Mlk1 Ml(Ru2)*
WBDC234	20 (50%)	10123605342771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*

WBDC235	2 (5%)	77767777757771	
WBDC236	4 (10%)	76727777775771	
WBDC237	0 (0%)	77777777777771	
WBDC238	13 (32.5%)	24777631036771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC240	15 (37.5%)	20567210677771	*Mla3*
WBDC241	0 (0%)	77777777777771	
WBDC242	7 (17.5%)	57377675276771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC243	3 (7.5%)	76727777777771	
WBDC244	29 (72.5%)	00100201022671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC245	0 (0%)	77777777777771	
WBDC246	0 (0%)	77777777777771	
WBDC247	6 (15%)	66767755776771	
WBDC248	33 (82.5%)	04000000000671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC250	3 (7.5%)	57377677777771	
WBDC252	10 (25%)	36727647176771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC253	1 (2.5%)	77777777377771	
WBDC254	1 (2.5%)	77767777777771	
WBDC255	0 (0%)	77777777777771	
WBDC256	13 (32.5%)	63173055366771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC257	2 (5%)	77777767677771	
WBDC258	1 (2.5%)	77777777777761	
WBDC259	0 (0%)	77777777777771	
WBDC260	0 (0%)	77777777777771	
WBDC261	0 (0%)	77777777777771	
WBDC262	2 (5%)	67767777777771	
WBDC263	5 (12.5%)	56727777775771	
WBDC265	23 (57.5%)	30100601332671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC266	8 (20%)	27777671276771	
WBDC267	4 (10%)	57777563777771	
WBDC268	1 (2.5%)	77767777777771	
WBDC269	2 (5%)	57777777776771	
WBDC270	19 (47.5%)	40004635475771	
WBDC271	14 (35%)	64367613356351	*Mla1 Mla6 Mla9 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC274	1 (2.5%)	77767777777771	
WBDC275	35 (87.5%)	00000000000670	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC276	0 (0%)	77777777777771	
WBDC277	14 (35%)	51767473252351	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlg Mlat Ml(Ru2)*

WBDC278	20 (50%)	20025470266771	*Mla12 MlLa Ml(Ru2)*
WBDC279	2 (5%)	77767777377771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC280	2 (5%)	77377677777771	
WBDC281	18 (45%)	24347671304351	*Mlk1*
WBDC282	3 (7.5%)	77747777776771	
WBDC283	2 (5%)	77377677777771	
WBDC284	5 (12.5%)	67747577377771	*Mla12 Mlat Ml(Ru2)*
WBDC285	2 (5%)	77577777677771	
WBDC286	16 (40%)	34605516364771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC287	1 (2.5%)	77767777777771	
WBDC288	1 (2.5%)	77777777377771	
WBDC289	35 (87.5%)	00001000044401	*Mla1 Mla3 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC290	29 (72.5%)	14440100000671	*Mlk1 Mlat*
WBDC291	34 (85%)	00000000000671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC292	23 (57.5%)	01124007352651	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC293	9 (22.5%)	01677477377771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC294	4 (10%)	53767777377771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC295	1 (2.5%)	77767777777771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC296	1 (2.5%)	77767777777771	
WBDC297	3 (7.5%)	77767777757761	
WBDC298	1 (2.5%)	77777777377771	
WBDC299	1 (2.5%)	77767777777771	
WBDC300	1 (2.5%)	77767777777771	
WBDC302	1 (2.5%)	77777757777771	
WBDC303	3 (7.5%)	77767577377771	
WBDC304	2 (5%)	77777577377771	
WBDC305	1 (2.5%)	77777777377771	*Mla12*
WBDC306	1 (2.5%)	77777777677771	
WBDC307	4 (10%)	76727777775771	
WBDC308	0 (0%)	77777777777771	
WBDC309	0 (0%)	77777777777771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC310	0 (0%)	77777777777771	
WBDC311	0 (0%)	77777777777771	
WBDC312	0 (0%)	77777777777771	
WBDC314	1 (2.5%)	77767777777771	
WBDC315	1 (2.5%)	77777777377771	
WBDC316	3 (7.5%)	76767777377771	

WBDC317	4 (10%)	75765777777761	
WBDC318	27 (67.5%)	46004004002771	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC319	0 (0%)	77777777777771	
WBDC320	1 (2.5%)	77777777377771	
WBDC323	16 (40%)	00000777777761	*Mla6*
WBDC324	0 (0%)	77777777777771	
WBDC326	3 (7.5%)	77777763377771	
WBDC329	1 (2.5%)	77777767777771	
WBDC330	4 (10%)	75757777777561	*Mla6*
WBDC331	15 (37.5%)	00000777777771	*Mla6*
WBDC332	4 (10%)	66777677377771	
WBDC333	5 (12.5%)	77767733367771	
WBDC334	4 (10%)	77747763777771	*Mla12 Mlg*
WBDC335	2 (5%)	77777577377771	
WBDC336	4 (10%)	67337777377771	
WBDC337	3 (7.5%)	77777557577771	
WBDC338	12 (30%)	67577571071721	
WBDC340	22 (55%)	76523422000661	*Mla3 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC341	25 (62.5%)	00275000041771	
WBDC342	4 (10%)	77767577657771	
WBDC343	4 (10%)	76777577357771	
WBDC345	9 (22.5%)	73753153377671	*Mla6 Mlk1 Mlat Ml(Ru2)*
WBDC346	31 (77.5%)	00000061045051	
WBDC347	15 (37.5%)	67325275145561	*Mlk1 Mlg*
WBDC348	28 (70%)	00202050024671	*Mla1 Mla6 Mlk1 Mlat Ml(Ru2)*
WBDC349	22 (55%)	63000615244671	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC350	0 (0%)	77777777777771	
WBDC354	33 (82.5%)	00000406000251	*Mla1 Mla6 Mla12 Mlk1 MlLa Mlat Ml(Ru2)*
WBDC355	16 (40%)	00000777677771	*Mla6*

[1]Wild Barley Diversity Collection accession number.
[2]Number of powdery mildew isolates out of 40 for which the WBDC accessions exhibited resistance (i.e. IT ≤1.5)
[3]Octal code reflects the resistance spectrum of each WBDC accessions to the whole panel of *Bgh* and is patterned after the system of 0004, 0020, 0023, 0061, 0235, 0323, 0331, 0574, 1002, 1044, 1377, 1541, 2567, 3707, 3777, 4404, 4523, 4611, 4761, 4776, 5137, 5425, 5511, 5735, 5765, 5715, 6000, 6040, 6045, 6737, 7377, 7557, 7737, 7777, H-148, J-462, Q-301, S-016, Y-035, Y-069
[4]Postulated resistance gene(s) present in the WDBC accessions based on the comparative infection types exhibited by the Pallas NILs.